SUMMARY

The thesis is presented in two parts with one appendix.

Part I was prompted by a report that the starch isolated from the seeds of Phalaris Canariensis possessed hypotensive activity. A review of the literature on Phalaris species is presented and the isolation of the starch and its extracts is described. No hypotensive activity of the starch or its extracts was found.

Chemical examination of aqueous extracts of the starch showed the presence of choline, glucose, maltose, azelaic acid and aminoacids. Ion exchange and paper chromatography were used to identify serine, α-alanine, phenylalanine, leucine, proline, aspartic acid, valine and aminobutyric acid.

The light petroleum soluble matter of the powdered seeds was examined and a mixture of sterols was isolated. β - sitosterol was identified in the mixture by conversion of the sterols to the methyl ethers and examination of the ethers by gas chromatography; two other components in the mixture were not identified. It is also shown that the "β - sitosterol" isolated from Aristolochia reticulata consisted of two components one of which is β - sitosterol. The fatty acids in the extract were identified as myristic, palmitic, stearic, oleic and linoleic acids, and glycerol was also isolated.

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Part II has as its main theme the attempted synthesis of aristolochic acid. A review of the degradation work carried out on aristolochic acids is given followed by an account of the pharmacological activity of extracts and compounds isolated from Aristolochia species.

After a report on the synthesis of derivatives of aristolochic acid, in which the benzoate of 8 - methoxy -3,4-methylendioxy-10-nitro-1-phenanthroic lactam was obtained as the only new product, a route to aristolochic acid is proposed. This was based on the Pschorr Synthesis, as one of the required intermediates, 2-nitro-6-methoxybenzaldehyde was known. The attempted preparation of the other intermediate, 6-carboxy-3,4-methylenedioxyphenylnitromethane, is discussed and the structure for a new compound obtained during this work is suggested. The lack of success in this stage turned attention to the synthesis of 3,4-methylenedioxyphenylnitromethane and the corresponding 6-bromo-compound and both compounds were obtained by two routes. One of these involved the use of metal nitrites and the conditions used and reactions taking place are considered in some detail.

The condensation of the nitro-compound with benzaldehyde and substituted benzaldehydes was investigated and the following compounds were prepared.

α-nitro-6-bromo-3,4-methylenedioxy-2'-nitro-6'-methoxystilbene.

α-nitro-3,4-methylenedioxy-2'-nitro-6'-methoxystilbene.

α-nitro-6-bromo-3,4-methylenedioxy-6′-methoxystilbene

α-nitro-6-bromo-3,4-methylenedioxy-2′-nitrostilbene.

α-nitro-3,4-methylenedioxy-2′-nitrostilbene.

Side reactions occuring during the condensation are discussed and tentative structures advanced for two of the products. Attempted condensation of acetylaminobenzaldehyde with the substituted phenylnitromethanes met with no success. Selective reduction of α-nitro-6-bromo-3,4-methylenedioxy-2′-nitrostilbene and α-nitro-3,4-methylenedioxy-2′-nitrostilbene by ammonium sulphide caused the elimination of one nitro-group from each compound and evidence is presented to show that the products were 6-bromo-3,4-methylenedioxy-2′-nitrostilbene and 3,4-methylenedioxy-2′-nitrostilbene respectively. The reduction of the nitro-compounds was also attempted by a variety of reducing agents, but no satisfactory product was isolated. In order to avoid selective reduction of a nitro-group irradiation of α-nitro-6-bromo-3,4-methylenedioxy-6′-methoxystilbene and 6-bromo-3,4-methylenedioxy-2′-nitrostilbene was attempted on the micro-scale, but ultraviolet absorption evidence indicated that the phenanthrene nucleus was not formed.

Part II concludes with an assessment of the results obtained and a scheme for further work is proposed.

The appendix presents the results of an examination of extracts of small quantities of the roots and rhizomes of A. goldieana and of the aerial parts of A. indica. No aristolochic acid was isolated from the extracts.

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STUDIES ON

THE CHEMISTRY OF *PHALARIS CANARIENSIS* AND OTHER NATURAL PRODUCTS

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Finally he wishes to express his sincere thanks to his parents and brothers for their full financial assistance.

C O N T E N T S

PART I. STUDIES ON THE CHEMISTRY OF PHALARIS CANARIENSIS

PART II ATTEMPTED SYNTHESIS OF ARISTOLOCHIC ACIDS

PART I

STUDIES ON THE

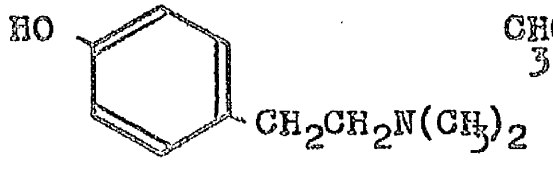
CHEMISTRY OF *PHALARIS CANARIENSIS*

HISTORICAL INTRODUCTION

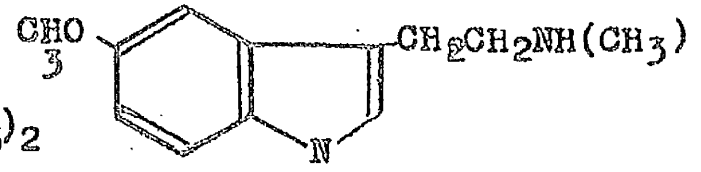
The genus Phalaris, which is the ancient Greek name for grass, belongs to the family Gramineae and contains about twenty species[1] of annual or perennial grasses. It is widely distributed in southern Europe, temperate America,[2] the Mediterranean region and Central Asia[3], and is much cultivated in Europe for canary seeds. There are no detailed chemical analyses of Phalaris species as investigations have been confined to products obtained by particular extraction procedures. In fact, the only general account of chemical composition is that of Lomanitz[4] for the leaves of one of the species P. bulbosa, but even here the results are given in general terms as indicated in Table 1 (page 7). Consequently a review of the work done is conveniently discussed by considering various groups of chemicals. Phalaris species are a potential source of foodstuff, and the protein content, and hence the aminoacids, have been investigated by a number of workers. The most recent report is that of Schneider[5] who identified a large number of aminoacids in an extract of the leaves of P. arundinaceae by paper chromatography using phenol-water (75:25) as solvent (Table 1). He compared his results with those of earlier workers[6,7,8] among whom Lugg and his co-workers have been particularly active in this field. Lugg[9] described the method of preparation of protein of the leaves of P. tuberosa; the amide, cystine (and or cysteine), and methionine[10,11] contents in the hydrolysate of the protein preparation

were estimated chemically. Tyrosine and tryptophan were also estimated chemically by the method described by Lugg[12,13] as the Folin and Ciocalteu[14] method for the estimation of tyrosine was found to be unsatisfactory. Lugg and Weller[15] determined the content of arginine and lysine in the protein hydrolysate from the leaves of P. tuberosa by the method described by Tristram[16]; histidine was determined by the method of Vickery and Winternitz[17] A modification of the Kapeller-Adler[18] colorimetric method of estimating histidine recommended by Block[19] was unsatisfactory. Gupta and Das[20] used microbiological methods to identify methionine, cystine and tryptophan in the hydrolysate of the extract of the leaves of P. tuberosa and P. canariensis.

Alkaloids have been reported in P. arundinaceae by Wilkinson[21] who isolated hordenine(I)and a new indole



(I)



(II)

alkaloid 5-methoxy-N-methyltryptamine(II.) The minced grass was extracted with ethanol, and the concentrated extract, diluted with water basified with sodium hydroxide solution, extracted with chloroform. Paper chromatography of the alkaloids with n-butanol-water-acetic acid (12:5:3) followed by spraying with dimethylaminobenzaldehyde in cyclohexane, and treatment with hydrogen chloride showed the presence of two indoles (R_F 0.58 and 0.70). The alkaloids were

separated by chromatography of the concentrated chloroform extract on alumina, the course of separation being followed by paper chromatography and colour reaction. Hordenine(I) was isolated from the first runnings, whilst the later fractions yielded a crystalline hydrochloride identified as 5-methoxy-N-methyltryptamine hydrochloride. The second minor indole alkaloid (R_F 0.70) was not identified because of its presence in small amount. The author reported that it was not gramine, but on a paper chromatogram it gave the same colour reaction and the same R_F value as 3-diethylamin-omethylindole.

The oil extracted from the seeds of P. canariensis by means of pressing and extracting with petroleum ether was examined by Olquellen[22]. Fatty acids and unsaponifiable matter were obtained, but their results (Table I) were expressed in general terms of saponification number, iodine number and appearance of the various fractions, and no identification of the components was made.

Pharmacological interest in Phalaris tuberosa lay in its possible value as a nutrient for birds and animals. Phalaris "staggers" in sheep and other animals have, however, been reported[23,24] by several authors. Thus Lee and Kuchel[25,26] reported that the staggers syndrome developed in Merino ewes when they were grazed on P. tuberosa. No symptoms were shown by ten ewes which were treated with 7 mg. of cobalt weekly, but eleven of fifteen untreated ewes were affected, six fatally. The response to cobalt

was highly significant, the animals being completely protected from this demyelinating disease. Similar effects were observed by Dewey, Lee, Dr. Hedley and Marston[27]. Walker[28] observed that sheep grazing on P. tuberosa developed nervous disorders, staggers, the symptoms of which were an initial hyperexcitability followed by muscular tremors, uneo-ordinated gait and stumbling or falling when driven. Demyelination in the spinal cord also occurred. Similar symptoms and pathological changes were observed in cats[29], dogs[30] and hens[31] treated with cholinesterase inhibitors. It would appear that other grasses are essential in pastures to dilute the P. tuberosa for grazing purposes.

The seeds of P. canariensis were used as a nutrient for birds and animals and have been employed as a poultice[32]. Rogazinski and Glowczynski[33] reported that seeds of canary grass (P. canariensis) are similar to the ordinary cereals in chemical composition and digestibility. They carried out experiments with white rats and produced rachitic symptoms due to the deficiency of calcium in the seeds, but when calcium was added to the diet rachitic symptoms did not appear. The authors concluded that in biological value the seeds were inferior to whole wheat. Glowczynski[34] further carried out experiment with chicks and showed that the seeds of canary grass must constitute 30-60 per cent of the diet in order to furnish a sufficient amount of vitamin A. In such quantities, however, the

diet may cause persistant diarrhoea.

Interest in the seeds of P. canariensis was renewed when the pharmacological laboratories of Smith Kline & French[35] confirmed a report of Dr. Pisanty[35] that the starch extracted from the seeds was hypotensive when administered to rats orally either in large doses or intravenously in small doses. The crude starchy preparation was unsuitable for injection both because of mechanical blocking of capillaries by large starch particles and the large doses required, and attempts were therefore made to obtain the active principles in a more suitable form. The starch, when treated with methanol in a Soxhlet extractor, was completely deactivated. Hydrolysis of the starch with dilute sulphuric acid or hydrochloric acid until negative iodine-potassium iodide tests were obtained also led to an inactive product, but boiling with water for six hours had no effect on the activity. An extract, prepared by boiling the starch with water, cooling and hydrolysing with α-amylase until a negative iodine-potassium iodide test resulted, gave a hard resinous material on evaporation which possessed full pharmacological activity. An attempt to extract an active principle from an enzymatically hydrolysed solution by continuous extraction with ether proved unsuccessful and full activity still remained in the aqueous phase.

In further attempts to concentrate and identify an active principle a nitrogen-free preparation was made.

The crude starch was boiled with water, and filtrate whilst hot to remove a dark coloured nitrogenous solid. The filtrate cooled to room temperature and treated with acetone, gave a completely inactive precipitate of nitrogen-free starch. The aqueous acetone solution after concentration in vacuo was found to be strongly active.

The active fraction appeared, therefore, to be soluble in water, but not in acetone. It did not contain sulphur or halogen, but it was nitrogenous and gave a positive reaction with ninhydrin and a weakly positive reaction with Mayer's and Hayer's solutions. Positive reactions were obtained with Benedict's solution and with phenylhydrazine, and alkali produced a deep yellow colour. The material fluoresced under ultraviolet light and gave a gelatinous precipitate with lead acetate. An unusual feature of the material was that precipitation of the starch with cold methanol instead of acetone gave rise to completely inactive products.

SUMMARY OF THE CHEMISTRY OF *PHALARIS* SPECIES

Table 1

Species	Part of Plant	Constituents	Remarks	Reference
P. canariensis	Seeds	Oil	5-6% bright yellow slightly aromatic. Saponification No. 184. Iodine No. 115.5. Acid value 20.8.	22
		Fatty acids	Saponification No. 203. Iodine No. 126.3.	
		Unsaponifiable matter	yellow waxy 1.5%	
		Choline		Present Work
		Azelaic Acid.		

Table I

CONTINUED

Species	Part of Plant	Constituents	Remarks	Reference
P. canariensis	Seeds	Aspartic acid		
		Proline		
		Leucine		
		Serine		
		α-Alanine		Present
		β-α-phenyl-alanine.		Work
		Valine		
		Amino-butyric acid.		
		Myristic acid		
		Palmitic acid		
		Stearic acid		
		Oleic acid		
		Linoleic acid		
		Glucose		
		Maltose		
		β-sitosterol		
		Two sterols	unidentified	

Table I

CONTINUED

Species	Part of Plant	Constituents	Remarks	Reference
P. canar-ensis	Seed Cake	Water	9.54 %	
		Oil	19.80 %	
		Albuminoids	17.35 %	
		Digestible Carbohydrates	38.50 %	36
		Woody fibre	8.67 %	
		Mineral matter	6.14 %	
		Sand and Silica	2.19 %	
		Food Units	1.31 %	
	Leaves	Methionine		
		Cystine		20
		Tryptophan		
P. bulbosa	Leaves	Pure protein	8.59 %	
	Dried at 100°	Amide	0.48 %	
		Digestible Protein	6.88 %	4
		Reducing sugars	1.86 %	
			2.22 %	
		Soluble starch and dextrin	1.31 %	
P. Tuberosa	Leaves	Methionine		20

Table I

CONTINUED

Species	Part of Plant	Constituents	Remarks	Reference
		Cystine		20
		Tryptophan		
P. tuberosa	Leaves	Amide	Nitrogen 4.76%	
		Cystine (and or cysteine)	Nitrogen 1.35%	10, 11
		Methionine	Nitrogen 1.46%	
		Tyrosine	Nitrogen 2.43%	
		Tryptophan	Nitrogen 2.04%	
		Arginine	Nitrogen 13.7%	15
		Lysine	Nitrogen 6.6%	
		Histidine	Nitrogen 13.66%	
P. arundinaceae	Leaves	Cystine		
		Serine		
		Glykocol		5
		Threonine		
		Alanine		
		Valine		
		Methionine		

Table I

CONTINUED

Species	Part of Plant	Constituents	Remarks	Reference
P. arundinaceae	Leaves	Leucine Isoleucine Proline oxy-proline Asparginic Acid Glutamic acid Lysine Arginine Histidine		5
	Leaves	Total crude protein	23.73%	37

D I S C U S S I O N

O F

E X P E R I M E N T A L

W O R K

STARCH

Isolation from seeds

The finely ground seeds were mixed with distilled water and part of the husk was removed by filtering the suspension through cheese cloth. After 36 hours in the presence of small quantities of streptomycin and papain about three quarters of the suspension was decanted, mixed and centrifuged to give four layers as described by Dr. Pisanty[35]. The starch layer was easily separated and dried over calcium chloride. This material as well as a batch of starch isolated by Smith Kline & French in the United States was used for the pharmacological and chemical tests.

Pharmacological Tests

An aqueous extract of the starch was prepared by heating with boiling water, cooling and precipitating the starch by the addition of acetone. The supernatant liquid on careful evaporation at room temperature under reduced pressure yielded a dark brown liquid which was injected into rats. No fall in blood pressure was observed over a period of 5 days. Oral administration of the starch itself failed to produce any fall in blood pressure. Similar negative results were obtained on repeating the experiments several months later.

Water-Soluble Bases

In view of the remarkably convincing evidence presented by the laboratories of Smith Kline & French on the hypotensive activity of Dr. Pisanty's[35] starch, it appeared desirable to examine the extracts chemically to try to identify material which could be associated in some way with hypotensive activity. In this respect two groups of compounds are of particular importance viz., alkaloids and aminoacids. Thus marked activity has been observed in the veratrine alkaloids[38] and also in the potent 5-hydroxytryptamine[39]. Tests were therefore instituted to detect compounds of these types. Tertiary bases were shown to be absent, but addition of ammonium reineckate[40] to the aqueous liquid remaining after extraction with chloroform and addition of acetic acid gave a bulky precipitate indicating the presence of one or more water-soluble quaternary ammonium bases. Decomposition of the reineckate was carried out with silver sulphate[41] but the base sulphate so obtained was extremely hygroscopic, as was the chloride; the picrate, however, proved satisfactory for characterisation of the base. The small quantity of material obtained from a relatively large amount of reineckate indicated that the basic component was of rather low molecular weight. Taken in conjunction with the melting point of the picrate (248-250°) choline was suspected and this was confirmed by comparison with authentic

material in the following tests: infrared absorption of choline reineckate and choline picrate, paper chromatography of the choline chloride[42] and choline reineckate[43] and mixed melting point of the picrates. The choline reineckate did not break up in the expected fashion unless the spot was treated with excess of silver nitrate[43] prior to chromatography, when silver reineckate gave a separate spot (R_F 0.42) while the liberated choline gave its characteristic spot (R_F 0.55). In view of the absence of other spots it would appear that choline is the only quaternary ammonium compound present and this would not give rise to hypotensive activity. The reineckate, as prepared above, was amorphous and required purification by column chromatography on alumina[44] using acetone as solvent. However, when the aqueous liquid remaining after the detection of carbohydrates (Page 37) was used, the reineckate separated as glistening crystals.

Aminoacids

Paper chromatography of aminoacids is an elegant technique for their identification and has long been used both in one dimension[45] and in two dimensions.[46] Preliminary experiments on the aqueous extract indicated the presence of a large number of compounds which gave a positive reaction with ninhydrin[47]. These compounds were confirmed as aminoacids rather than proteins or peptides by carrying out the simple hydrolysis procedure of Foster, MacDonald, and Jones[48] for the component aminoacids in ergometrine. The same number of spots with the same R_F value were found after this procedure as were found for the original solutions, indicating that the extract contained free aminoacids. The concentration of the acids was very small as judged by comparing the intensities of the spots with those obtained from standard solutions of aminoacids. It was, therefore, not considered feasible to attempt the isolation of the acids. It did appear necessary however to resort to some method of fractionation to assist in their identification.

Column chromatography on charcoal/celite[49,50] (1:2) gave a partial separation (Table 3), and reduced the number of components for paper chromatography at any one time. An ion-exchange technique[51,52] reported to be of considerable use for the separation of various groups of aminoacids[53], was applied to fractions A and B as it was hoped that sugars would be removed in this way.

The accurate comparison of the R_F values of the aminoacids in the starch extracts with authentic samples was therefore carried out on the fractions from the ion-exchange experiment. Each fraction was examined in two solvent systems, and each assignment was confirmed by the addition of authentic material and re-examination under the same conditions.

Aspartic acid, serine, α-alanine, leucine, proline, aminobutyricacid, valine and β- phenylalanine were identified in this way. From the results obtained by Moore and Stein[54] on the separation of aminoacids on an ion-exchange column arginine was expected in fraction A_4 and B_3 but the results were inconclusive. This may in fact be due to decomposition as considerable loss (30%) of arginine occurred unless the PH was carefully controlled. As Wilkinson[21] had isolated a tryptamine derivative, careful attention was paid to the possibility of tryptophan being present as a precursor.[39,55] However, it was not found.

CARBOHYDRATES

The concentrated aqueous extract (page24) was chromatographed on a column of charcoal/celite (1:2) using distilled water as solvent. Whistler and Durso[56] used a column consisting of equal quantities of charcoal and celite[57] to separate monosaccharides and disaccharides with distilled water and ethanol (5%) respectively. Charcoal columns have been used for purification[58] and separation[59,60,61] of sugars, but as adsorption effects are likely to be very strong with charcoal alone a mixed column was used in this work. Paper chromatography of the effluent using the upper phase of the solvent system n-butanol-acetic acid-water (4:1:5) indicated that two reducing substances were present in fraction A and one in fraction B. These effluents were examined chemically using phenylhydrazine, and by further paper chromatography[62] using samples of known sugars. Glucose was identified unequivocally by these means, but the compound which gave rise to the spot of R_F 0.47 could not be identified or separated from the glucose in spite of further column chromatography[63,64]. It did not appear to correspond to any sugar as even the pentose and tetrose sugars examined did not run as fast. It must be remembered, however, that the aqueous extract did not contain sugars only, but at least amino acids also.

Further elution of the column with ethanol (5%) in water yielded more glucose (fraction C). When ethanol (10%)

was used a different reducing sugar of R_F 0.11 was obtained. Paper chromatography using standard sugars, and osazone[65] formation, identified the sugar as maltose. The separation of monosaccharides and disaccharides was, therefore, easily accomplished on this column and the rather longer method of preparation of acetates followed by column chromatography[67] proved unnecessary. It would appear that glucose and maltose were the only sugars present as in addition to the silver nitrate technique for the detection of reducing sugars[68], chromatograms were also prepared for spraying with *p*-anisidine hydrochloride[69] in n-butanol. This reagent is useful in that it gives a cherry-red colour with pentoses, a green to brown colour with aldohexoses and a yellow colour with ketohexoses. The column was further developed with ethanol 5%, 20% 50%, 95% and N hydrochloric acid. Of these, only fraction F (Table 6, page 38) appeared of interest.

Azelaic Acid

Fraction F (page 38) yielded crystals which melted at 104-105° after recrystallisation. The infrared absorption curve indicated a carboxylic acid and azelaic acid was identified by comparison with authentic azelaic acid by infrared absorption, mixed melting point and paper chromatography using liquified phenol as solvent, and bromocresol green[70] as detecting agent.

The origin of the azelaic acid may well lie in an oxidation of oleic acid which was shown to be present in

the oil (page55). Curiously enough, fraction E which was thought to be impure azelaic acid, gave the reactions of calcium, phosphate and oxalate.

This concluded the chemical examination of the starch. The compounds identified could easily be adsorbed on the starch during its isolation and preliminary experiments on the supernatent liquid which was rejected (page24) indicated that carbohydrates and aminoacids were present. It is unlikely that an important base was lost at this stage because the solution failed to react with Dragendorff's reagent.

Many of the chemical results reported for the "active fraction" can be explained on the basis of the above analysis. Thus aminoacids account for the positive reaction with ninhydrin, glucose for the reaction with Benedict's solution, phenylhydrazine and the yellow colour with alkali and the other positive reactions imply no particular compound in view of the complexity of the extract. For example the bulky amorphous precipitate with ammonium reineckate is not accounted for by the presence of the choline alone, but may well be due to a salting out effect. In partially purified extracts the precipitate was smaller in bulk, but highly crystalline.

LIGHT PETROLEUM SOLUBLE MATTER OF SEEDS OF PHALARIS CANARIENSIS

As so little was known of the constituents of the oil from the seeds of P. canariensis, the availability of the seeds made it worthwhile to undertake a chemical examination of the oil. The seeds were finely ground and extracted by cold percolation with light petroleum until the percolate was colourless. Evaporation of the solvent yielded a yellowish green oil which did not deposit any solid on cooling or yield any volatile matter on steam distillation. The oil was, therefore, saponified with ethanolic potassium hydroxide and separated into unsaponifiable matter and fatty acids by the normal method.

Unsaponifiable Matter

Very little material was extracted by light petroleum from the soap solution and extraction with ether was necessary. The ether-soluble unsaponifiable matter was chromatographed on an alumina column using the solvents and mixtures of solvents detailed in Table 11. Of the twelve fractions collected, only fraction E, which contained crystalline material, was examined.

Repeated crystallisation of the solid from ethanol yielded fine white needles m.p. 136 -138° $[\alpha]_D^{20}$ -24, which gave reactions characteristic of β-sitosterol. The melting point and specific rotation indicated that the crystals were probably still a mixture and this was confirmed by

acetylation to a product which possessed a wide melting range (85 -135°).

Comparison of the infrared absorption spectrum of the crystals with that of "β-sitosterol" isolated from A. reticulata by Williams[71] showed a marked similarity, but not complete identity. This difference still persisted after crystallisation of both specimens from the same solvent and under the same conditions, a treatment which, it was hoped, would eliminate solid-state differences.

Confirmation, that the crystals were indeed a mixture, was sought by gas chromatography, but the sterol and its acetate failed to emerge from a column of 0.5% apiezon L on celite at 230°. Clayton[72] used the methyl ether for this type of work, and comparison of the sterol methyl ether with authentic β-sitosterol methyl ether (prepared from a sample of pure β-sitosterol m.p. 139-140° kindly supplied by Prof. Sorm) confirmed its non-homogeneity. The traces obtained (figure1) show two components in the ether prepared from Williams "β-sitosterol", three components in that from Phalaris canariensis and two in that from Professor Sorm's sample. β-Sitosterol is certainly present but in the absence of samples of other sitosterols it was not possible to identify the second component. The early appearance of the third component would suggest a compound of lower molecular weight than that of the sitosterols. Differences observed in the infrared absorption spectra are probably due to the presence of this third component.

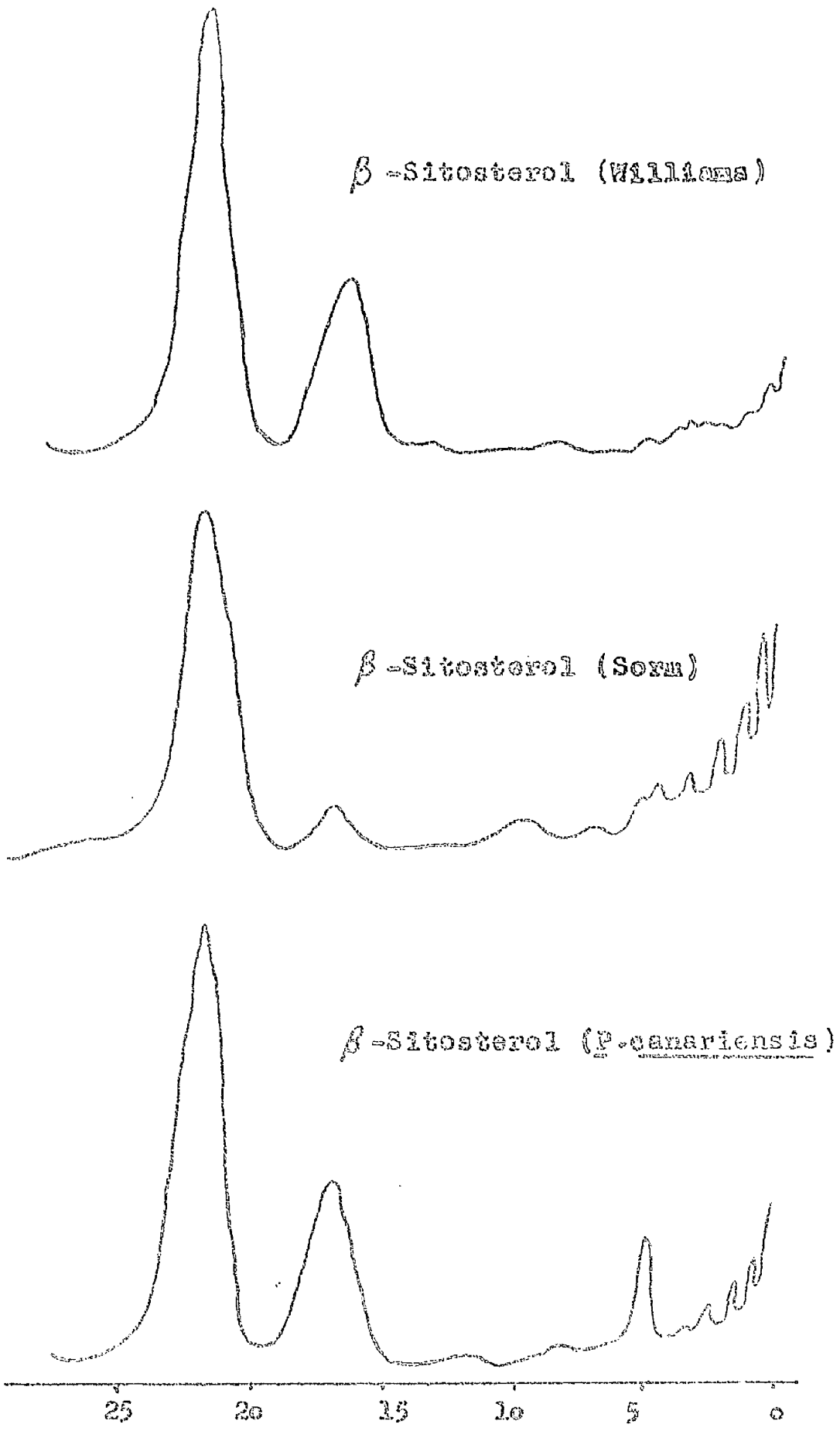

β -Sitosterol (Williams)
β -Sitosterol (Sorm)
β -Sitosterol (P. canariensis)
25
20
15
10
5
0
Minutes

Fig. 1.

The results of this examination clearly show the inadequacies of identification of natural products by purely chemical means.

Fatty Acids

Fatty acids were easily separated into saturated and unsaturated acids by the method of Twitchell[73] as modified by Hilditch[74]. The saturated acids were converted to the methyl esters as a pale yellow solid m.p. 30-35°, which unfortunately was in too small a quantity to be subjected to fractional distillation. The proportion of unsaturated esters was very small (iodine value 4.8) and the saponification value indicated the presence of acids up to C18. Methylpalmitate, methyl stearate and a trace of methyl-myristate were confirmed by gas chromatography using authentic esters.

The unsaturated acids were recovered from the soluble lead salts after the removal of saturated acids, and converted to methyl esters. These esters were fractionally distilled and the fractions examined to give the results summarised in Table 12. Linoleic acid was confirmed by the preparation of crystalline tetrabromostearic acid m.p. 113-115°, identical in m.pt., mixed m.pt., and infrared absorption spectrum with that of an authentic sample. The mother liquor of this fraction gave a small amount of semi-solid crystalline material which was not investigated further.

Oleic acid was confirmed in fraction (B) by the preparation of a crystalline dihydroxystearic acid by the

method of Scanlan and Sworn[75], m.p. 90-91° undepressed on admixture with an authentic sample. Both oleate and linoleate were confirmed by gas liquid chromatography of the methyl esters.

Glycerol was identified by evaporation of fraction E, when the residue gave the colour reaction[76,77] of glycerol and a crystalline tri-*p*-nitrobenzoate, m.p. 190-192°, undepressed on admixture with an authentic sample.

E X P E R I M E N T A L

STARCH

Isolation of Starch[35]

The finely ground seeds (1kg) were mixed with distilled water (1.5L) and allowed to stand for one hour. The mixture was filtered through three layers of cheese-cloth and the husk retained on the filter was discarded. Streptomycin (200 mg) and papain (90 mg) were added to the suspension which was maintained at room temperature for 36 hours. Approximately three quarters of the liquid was decanted, mixed and centrifuged at 2500 revolutions per minute. Four layers were formed, an aqueons dark brown supernatent (reported below) a fine resinous mass, starch and husk. The starch was separated and dried over calcium chloride in a desiccator, Yield 170 gm (17%).

The aqueous dark brown supernatent liquid gave positive tests for carbohydrate and amino acids, but no reaction for alkaloid with Dragendorffs reagent. The liquid was not examined further.

Preparation of extract for pharmacological tests.[35]

Freshly prepared starch (100 g) was heated to boiling with distilled water (1500ml) for one hour. The mixture was cooled in an ice-bath to below 20° and acetone (1500 ml) was added with continuous stirring, the temperature being maintained below 20°. Starch was precipitated and the light brown supernatent liquid was decanted. The starchy residue was twice stirred thoroughly with a mixture of an equal quantity

of acetone and water (300 ml) and allowed to settle. The combined supernatent liquid was evaporated to small volume (20 ml) under reduced pressure at about room temperature. The aqueous extract was allowed to stand overnight in a refrigerator, and the dark, clear, brown liquid was filtered.

Pharmacological examination(78)

The extract prepared as described above was injected into rats, but no fall in blood pressure was observed over a period of 5 days. Similar results were obtained with two different batches of starch and with oral administration of starch itself. The whole procedure was repeated with fresh extracts after several months with the same results.

CHEMICAL EXAMINATION OF STARCH EXTRACT

An extract was prepared from 1000 g. of starch in the manner described under "preparation of extract for pharmacological tests" (page 24) using appropriate volumes of solvents.

General tests on extract

Lead acetate	White fluffy precipitate.
Ferric chloride	No reaction.
Iodine	Precipitate.
Picric acid	Precipitate.
Phosphotungstic acid	Precipitate.
Ammonium reineckate	Buff coloured precipitate.
Ninhydrin	Light blue colour reaction.
Molisch's reagent	Purple violet ring formed.
Fehling's solution	Reduced.

Benedict's solution ... ... Reduced.

Bial's test... ... ... Green colour formed.

Seliwanoff's[79] test ... ... No reaction.

Millon's test ... ... White precipitate.

A few drops of the extract when treated with a solution of p-dimethylaminobenzaldehyde[21] in 65% V/V sulphuric acid (1 ml) gave the following colour sequence; light pink gradually changing to brown, greenish blue and finally (overnight) light blue.

Chloroform-soluble material

The concentrated extract (30 ml) prepared from starch (1000 g) was made alkaline with dilute solution of ammonia and extracted with chloroform (40 ml x 3). The combined chloroform extracts were washed with water, dried ($Na_2 SO_4$) and evaporated to yield a trace of resinous material (10 mg). It did not give any reaction for alkaloids and was not investigated further.

Water-soluble bases

The aqueous alkaline solution from the previous test was acidified with dilute acetic acid and excess of ammonium reineckate solution was added. The mixture was allowed to stand overnight, centrifuged and the clear supernatent liquid was decanted. The residue was washed with cold water and dried in a vacuum desiccator to give a buff coloured powder (2 gm) m.p. 254-257°. The reineckate (250 mg) was dissolved in acetone (5 ml) and treated with 0.6% silver sulphate[41] solution till no further precipitate was obtained. The mixture was filtered and the precipitate of silver reineckate was washed with aqueous acetone. The combined filtrate and washings were evaporated to dryness under reduced pressure and the residue was dried over P_2O_5 The residue was hygroscopic and to its solution in water was added a small excess of barium chloridesolution and the precipitate of barium sulphate was filtered off. The filtrate was evaporated and dried over P_2O_5 in a desiccator. The residue was very hygroscopic and could not be recrystallised. The chloride was dissolved in water (1ml) and a saturated solution of picric acid in ethanol was added to give an immediate yellow crystalline mass which was recrystallised from hot ethanol m.p. 248-250°.

Paper chromatography of the chloride and reineckate.

The base chloride and base reineckate along with choline chloride , choline reineckate and betaine hydro-

chloride as controls were chromatographed on Whatman No. 1 paper overnight using n-butanol-acetic acid-water (100:30:85, solvent I) and also (in a separate experiment) ethanol-ammonia[42] (95:5, solvent II). The spots of base reineckate and choline reineckate[43] were treated with a drop of aqueous silver nitrate solution before development. The chromatogram was dried in an oven at 60° and the compounds were detected with dilute solution of Dragendorff's[81] reagent and iodine vapour. The results are recorded in Table 2.

Table 2

Sample	R_F (solvent I)	R_F (solvent II)
Base chloride	0.55	0.46
Choline chloride	0.55	
Base reineckate (untreated)	0.54	0
Choline reineckate (untreated)	0.54	0
Base reineckate	(i) 0.42	(i) 0.16
(Treated with silver nitrate)	(ii) 0.55	(ii) 0.32
Choline reineckate	(i) 0.42	(i) 0.16
(Treated with silver nitrate)	(ii) 0.55	(ii) 0.32
Betaine hydrochloride	0.51	

Confirmation of Choline.

Choline was confirmed by the preparation of authentic choline picrate and choline reineckate, and comparison of infrared absorption curves and determination of mixed m.pts;

choline reineckate mixed m.p. 255-257° (decomp.) (undepressed)

choline picrate mixed m.p. 248-250° (undepressed).

Adsorption Chromatography

The concentrated aqueous extract (15 ml) from 1000 g of starch was chromatographed on a charcoal/celite column (1:2, 16"x1½"). The column was eluted with water and 100 fractions each of 10 ml, were collected by means of an automatic fraction collector. Elution of the column was continued with ethanol (20% 1000 ml), ethanol (50% 1000 ml) and ethanol (500 ml), each of these fractions being evaporated to small volume. Each fraction was examined by paper chromatography using liquified phenol[82] as solvent, 2% ninhydrin[47] as detecting agent, and the sequential fractions showing the same number of spots on the chromatogram were combined as shown in Table 3.

Table 3

Fractions	Combined as	Number of spots
0-20	-	-
21-32	A	6
33-50	B	7
51-100	C	3
20% ethanol	D	5
50% ethanol	E	4
ethanol	-	-

Attempted hydrolysis of fractions

Portions of Fractions A, B, C, D and E were hydrolysed by the method of MacDonald, Foster and Jones[48], each mixture was

made alkaline by excess of ammonia, and evaporated to dryness on a water bath. Water was added and each solution was filtered. Each filtrate was chromatographed on paper and developed in the same way as described above. The chromatograms showed the same number of spots and the same R_F values as did fraction A, B, C, D and E.

Attempted separation by ion-exchange.

A column of Zeocarb (51) 225 (Na form, 9"x1") was washed with N HCl (200 ml) followed by distilled water until neutral. N sodium hydroxide was added to the column until the eluate was alkaline to litmus paper followed by water till the washings were neutral.

Fraction A was reduced to small volume (3 ml) and added to the column which was eluted with water in 10 ml fractions followed by ammonia (10% 100 ml). Each fraction was examined by paper chromatography using (I) liquified phenol and (II) butanol-acetic acid-water(83) (4:1:5) as solvents and the eluate fractions were combined (Table 4) according to the appearance of the chromatograms. Fraction B was treated in a similar manner and the results are shown in Table 4.

Table 4

Fractions	Solvent I R_F values	Solvent II R_F values.
A1	0.11	
	0.19	0.13
	0.23	0.16

Table 4

CONTINUED

Fractions	Solvent I R_F values	Solvent II R_F values.
A_1	0.30	0.22
	0.40	
A_2	0.12	0 10
	0.18	0.12
	0.23	0.15
	0.40	0.20
	0.50	0.24
	0.60	0.43
	0.71	0.60
A_3	0.11	0.06
	0.19	0.12
	0.22	0.15
	0.31	0.21
	0.40	0.57
A_4	0.40	0.07
(NH_4OH)	0.50	0.27
B_1	0.10	0.09
	0.16	0.13
	0.21	0.18
	0.32	0.23

Table 4

Fractions	Solvent I R_F values	Solvent II R_F values.
B_1	0.38	0.32
	0.59	0.44
	0.69	0.58
B_2	0.38	0.34
	0.55	0.43
B_3 (NH_4OH)	0.36	0.10
	0.50	0.30

Identification of aminoacids.

Fractions A_1-A_4, B_1, B_2, B_3, C, D and E were submitted to paper chromatography using Whatman No. 1 paper and the descending technique with liquified phenol and n-butanol-acetic acid-water[83] (4:1:5) as solvents. Standard aminoacid samples were used at the same time and comparison of the R_F values of the sample and standard spots obtained with bothsolvents enabled the following aminoacids to be identified.

Table 5

Fraction	Aminoacids	R_F values Solvent I	Solvent II
A_1	Aspartic acid	0.13	
	Serine	0.23	0.15
		0.30	0.20
		0.38	

Table 5

CONTINUED

Fraction	Aminoacids	R_F values.	
		Solvent I	Solvent II
	α-alanine	0.50	0.26
A_2	Aspartic acid	0.12	0.10
		0.18	0.12
	Serine	0.23	0.15
		0.40	0.20
	α-alanine	0.50	0.25
		0.60	0.43
	Leucine	0.71	0.60
A_3	Aspartic acid	0.11	0.06
		0.19	0.12
	Serine	0.22	0.15
		0.31	0.21
		0.40	0.57
A_4		0.40	0.07
		0.50	0.27
B_1	Aspartic acid	0.13	0.09
	Serine	0.22	0.13
		0.30	0.18
		0.40	0.23

Table 5

CONTINUED

Fraction	Aminoacids	R_F values Solvent I	Solvent II
B_1	α-alanine	0.50	0.32
	Aminobutyric acid	0.70	0.44
	Proline (yellow)	0.80	0.58
B_2	Aminobutyric acid	0.70	0.47
		0.36	0.10
$B_3(NH_4OH)$		0.50	0.30
C	Aspartic acid	0.12	0.11
	Valine	0.67	0.37
	Leucine	0.82	0.61
D	Aspartic acid	0.13	0.1
	Serine	0.21	0.19
		0.29	0.23
	α-alanine	0.50	0.39
	Leucine	0.79	0.59
E	Aspartic acid	0.14	0.09
		0.30	0.31
		0.39	0.39
	β-phenylalanine	0.56	0.56

(ii) The paper chromatography was repeated under the same conditions, but with the addition of authentic samples of aminoacids to the appropriate fraction. The same number of spots were obtained as in the previous experiment (i) above.

Compounds with R_F value of 0.18, 0.30, 0.40 and 0.60 (liquified phenol) were not identified.

Carbohydrates

The concentrated aqueous extract (20 ml) from 1000 g of starch was chromatographed on a column of charcoal/celite (1:2 16" x 1½"). Each fraction collected was 50 ml and fractions 1-32 were examined by paper chromatography (see below). The change over from one solvent to the next occurred when the response to Molisch's test became very weak. The results are recorded in Table 6.

Table 6

Fraction	Solvent	Remarks	R_F value
	Water	Two components.	0.182
1-4		These fractions were combined and evaporated to small volume (2 ml). Fraction 'A'.	0.47
5-12		One component. These fractions were combined and evaporated to small volume (3 ml) Fraction 'B'.	0.182
13-20	5% ethanol in water	One component. These fractions were combined and reduced to small volume (2ml). Fraction 'C'.	0.181

Table 6

CONTINUED

Fraction	Solvent	Remarks	R_F Value
21-32	10% ethanol in water	one component Fraction 'D'	0.112
33-38	15% ethanol in water	These fractions gave a very weak Molisch's test, but on concentration deposited white crystalline material. Fraction 'E'	
39-44	20% ethanol in water.	These fractions gave a very weak Molisch's test, but on concentration deposited white crystalline material. Fraction 'E'	
45-50	50% ethanol	No response to Molisch's test, but glistening crystals were found m.p. 104-105° Fraction 'F'.	

Table 6

CONTINUED.

Fraction	Solvent	Remarks	R_F Value
51-60	95% ethanol	A trace of sticky oil was found which did not give a positive reaction for carbohydrate or alkaloid. It was not investigated further.	
61-70	N Hydrochloric acid	These fractions on evaporating deposited a trace of resin and were not investigated further.	

Chromatography

Fractions 1-32 were examined by paper chromatography (descending technique) using as solvent, the upper phase obtained by shaking n-butanol-acetic acid-water[84] (4:1:5) and allowing to separate. The lower layer was used to saturate the atmosphere in the chromatography tank. Detection of the components were carried out spraying with an acetone solution of silver nitrate[85] drying in air followed by spraying with alcoholic soduim hydroxide and drying at 60°. The dark brown background was removed with 20% sodium thiosulphate. The results are

recorded in Table 6 which also shows which fractions were combined to give fractions A, B, C, D, E and F.

Fractions 'A' 'B' 'C' 'D' were examined by paper chromatography using solvent n-butanol-acetic acid-water (4:1:5) and compounds were detected by 3% p-anisidine hydrochloride[69] in n-butanol. The results are recorded in Table 7.

Table 7

Fraction	Colour	R_F value	Remarks
A	Yellowish brown	0.182	Glucose
	Light pink	0.47	not identified
B	Yellowish brown	0.182	Glucose
C	Yellowish brown	0.181	Glucose
D	Yellowish brown	0.112	Maltose
Glucose	Yellowish brown	0.182	
Fructose			
Galactose	Yellowish brown	0.17	
Maltose	Yellowish brown	0.11	
Lactose	Yellowish brown	0.08	
Arabinose	Cherry red	0.22	
Xylose	Cherry red	0.27	
Rhamnose	Cherry red	0.36	

Table 7

CONTINUED

Fraction	Colour	R_F value	Remarks
Mannose	cherry red.	0.20	
Ribose	cherry red.	0.30	

The spray system (page 39) was also used to detect the components, and fructose in this experiment gave an R_F value of 0.22.

Fraction 'A' was chromatographed on charcoal/celite (1:2, 8" x 1"). The column was eluted with distilled water (200 ml), each fraction being 10 ml. The results are recorded in Table 8.

Table 8

Fraction	R_F value	Components	Remarks
1			
2			
3			
4			
5	0.182, 0.47	Two components	
6	0.182, 0.46	Two components	No separation.
7	0.182, 0.47	Two components	Combined as
8	0.182, 0.47	Two components	fraction A_1.
9	0.182	One component	
10	0.182		
11	0.182		
12	0.182		

Table 8

CONTINUED

Fraction	R value	Components	Remarks
13	0.182	One component	
14	0.182		
15	0.182		
16	0.182		
17	0.182		
18			
19			
20			

Fraction 5-8 were combined as fraction 'A_1'.

Fraction 'A_1' was chromatographed on cellulose (16 cm x 14 mm) soaked with the lower phase of the solvent system n-butanol-ethanol-water (4:1:5)[86] and the column was eluted with upper phase (160 ml). The volume of each fraction was 5 ml. The results are recorded in Table 9.

Table 9

Fraction	R_F value	Components
1		
2		
3		
4		
5	0.182, 0.47	Two components.
6	0.182, 0.47	Two components.
7	0.182, 0.46	Two components.

Table 9 CONTINUED

Fraction	R_F value	Components
8	0.182, 0.47	Two components
9	0.182, 0.47	Two components
10	0.182	One component
11	0.182	One component
12	0.182	One component
13	0.182	One component
14	0.182	One component
15	0.182	One component
16	0.182	One component
17	0.182	One component
18	0.182	One component
19	0.182	One component
20	0.182	One component
21	0.182	One component
22	0.182	One component
23	0.182	One component
24	0.182	One component
25	—	—
26	—	—
28	—	—
29	—	—
30	—	—

Osazones(65)

Fraction 'A' (1ml) was heated with phenylhydrazine hydrochloride (0.5g) and anhydrous sodium acetate (0.2g) on

boiling water bath for ten minutes. Mixed crystals of osazones were deposited, the major proportion being clusters of long slender needles.

Fractions 'B' and 'C' (each of 1 ml) were heated with phenylhydrazine hydrochloride (0.5 g) and anhydrous sodium acetate (0.2g) on a boiling water bath for five minutes. A cluster of long slender needle-like crystals separated m.p. 201-204°, mixed m.p. with authentic glucosazone was undepressed.

Fraction 'D' (1 ml) was heated with phenylhydrazine hydrochloride (0.5 g) and anhydrous sodium acetate (0.2g) on a boiling water bath for ten minutes. Long rod-like crystals were deposited m.p. 198-201°, undepressed on admixture with authentic maltosazone.

Azelaic Acid

Fraction F (Table 6, page 38) were recrystallised from ethanol 50% as shining flakes (38 mg) m.p. 104°, infrared absorption 1690 cm^{-1} (COOH) identical in all respects with the infrared absorption curve of authentic azelaic acid. Mixed melting point with authentic azelaic acid was undepressed.

The acid was confirmed by paper chromatography (ascending technique) along with authentic azelaic acid and other acids as controls, using liquified phenol as solvent and bromocresol green[70] as developing reagent. White spots were present on green background. The results are recorded in Table 10.

Table 10

Compounds	R value
Malic acid	0.40
Lactic acid	0.80
Citric acid	0.26
Tartaric acid	0.18
Glutaric acid	0.81
Oxalic acid	0.40
Succinic acid	0.63
Sample	0.74
Azelaic acid (authentic)	0.75
Calcium lactate	0.71
Adipic acid	0.33

The crystals (fraction E) were not, as expected, impure azelaic acid, but gave positive reactions for calcium, oxalate and phosphate. They were not examined further.

THE LIGHT PETROLEUM SOLUBLE FRACTION OF THE SEEDS OF PHALARIS CANARIENSIS

Extraction of seeds

Freshly ground seeds (550g) were extracted in a Soxhlet extractor with boiling petroleum on a water bath until the solvent in the siphon was colourless. The extract was cooled, filtered and evaporated under reduced pressure on a water bath to yield a semi-solid yellowish green extract (30g 5.45%). It was kept in a refrigerator overnight, but there was no deposition of crystalline material.

Steam distillation of Extract.

The semi-solid extract (30g) was melted in a flask with distilled water (300 ml) and steam distilled for two hours. The distillate was extracted with light petroleum (50 ml x 3) and the petroleum extracts were bulked, washed with water, dried (Na_2So_4) and the solvent was removed under reduced pressure. There was no residue.

Unsaponifiable Matter

The non-volatile residue (28g) from the steam-distillation was refluxed with ethanolic potassium hydroxide (15 gm of potassium hydroxide in 100 ml of ethanol) on a boiling water bath until solution occurred (1½ hours) and for a further three hours. The ethanol was removed under reduced pressure, water being added to maintain the volume constant. The soap solution was cooled, diluted with water (100 ml) and extracted with light petroleum (200 ml x 3). The petroleum extracts were bulked, and

washed with water (50 ml x 2), the washings being added to the soap solution. The petroleum extract was dried and the solvent removed under reduced pressure to give a trace of golden yellow oil which was not investigated further. The soap solution was extracted with diethyl ether (200 ml x 3), the ether extracts were bulked, washed with water (50 ml x 2), the washings being added to the soap solution and dried (Na_2So_4). The ether was removed to give unsaponifiable matter as a light golden yellow semi-solid residue (0.4g). The aqueous residue was reserved for the examination of fatty acids.

Chromatography of Unsaponifiable Matter.

The residue (0.4g) was dissolved in benzene (10 ml) and chromatographed on an alumina column (9" x 1") which had been previously washed with benzene.

The results are recorded in Table 11.

Table 11

Fraction	Solvent	Volume	Residue
A	Benzene	200	Trace of yellow oil.
B	Benzene + 10% ether	200	Trace of yellow oil.
C	Benzene + ether(1:1)	200	Trace of yellow oil.
D	Benzene & Ether (1:1) with 1% ethanol.	150	Trace of yellow oil.

Table II

CONTINUED.

Fraction	Solvent	Volume	Residue
E	Benzene & Ether(1:1) with 2% ethanol.	300	White crystalline material (75 mg) containing some colouring matter.
F	Benzene & Ether(1:1) with 5% ethanol.	200	Trace of yellow oil.
G	Benzene & Ether(1:1) with 10% ethanol.	150	Trace of yellow oil.
H	Benzene & Ether(1:1) with 20% ethanol	100	None.
I	Benzene	300	None.
J	Ether	300	None.
K	Ethanol	150	None.
L	Petroleum Ether	200	None.

Isolation of Sterols

On repeated crystallisation of fraction E from hot ethanol fine, white needles m.p. 136-138° were obtained, $[\alpha]_D^{20}$ - 24, (c = 1 in chloroform).

The crystals gave positive reactions with the Liebermann Burchard[88], Salkowski[89], Rosenheim[90], Mach[91], Moleschotts[91] and Zimmerman[92] reagents.

Preparation of Sterol Acetates The sterols (20 mg) were

heated with acetic anhydride (0.2 ml) for two hours on a boiling water bath. The mixture was diluted with ethanol (1 ml) and heated for 30 minutes under reflux. The solution was diluted with water and cooled in a freezing mixture. The crystalline material (10 mg) was filtered off and re-crystallised from ethanol as glistening flakes, which softened at 85° and melted completely at 135°.

Preparation of sterol methylethers. The sterols (2 mg) were refluxed with one drop of methyl iodide in ether (15 ml) in the presence of a small quantity of potassium-tertiary-butoxide for 4 hours. The mixture was extracted with water (10 ml x 2) and the ether layer was dried (Na_2SO_4) The solvent was evaporated to give a white crystalline residue.

Gas chromatography of the Sterols and their derivatives. The compounds were submitted to gas liquid chromatography on a Pye Panchromatograph under the following conditions.

Column: Apiezon L. 0.5% on celite 100-120 mesh(5 ft)

Temperature: 228°

Gas: Argon.

Gas flow: 100 ml/minute.

Detector: β-ionisation

Detector temperature: 240°

Sensitivity: 1×10^{-8}

Solution: 5% in cyclohexane (2 μl)

Both the sterols and its acetate failed to emerge, but the methyl ether was entirely satisfactory. The result is recorded in figure I.

Samples of β-sitosterol obtained from Williams[71] and from Professor Sorm were converted to the methyl ether and subjected to chromatography as described above. The results are recorded in figure I.

Fatty acids

Isolation Acidification of the reserved aqueous liquid (Page 47) with dilute hydrochloric acid decomposed the soaps and precipitated the fatty acids as a light yellow semi-solid. It was extracted with light petroleum (200 ml x 3), the petroleum extracts were bulked, washed with water (50 ml) the washings being added to the aqueous solution, dried (Na_2So_4) and the solvent was removed to give a brownish yellow semi-solid (15g). The aqueous layer was further extracted with ether (200 ml x 3), the ether extracts were bulked, washed with water (50 ml), the washings being added to the aqueous solution, and dried (Na_2So_4). The solvent was evaporated to give a trace of yellow oil which was not investigated further. The aqueous layer was reserved for the examination of glycerol.

Saturated Acids.

Isolation of lead salts. The acids (15g) were dissolved in ethanol (95% 75 ml) and the solution was heated to boiling. A boiling solution of lead acetate (10g) in ethanol (70 ml) containing 2 ml of acetic acid was added and the mixture was cooled overnight. The white crystalline precipitate was filtered off, washed with 95% ethanol and recrystallised twice from 95% ethanol (40 ml) which contained a small amount

of glacial acetic acid. White crystalline lead salts of the saturated fatty acids were obtained m.p. 98-102°.

The combined filterate and washings were reserved for the examination of the unsaturated acids.

Decomposition of lead salts. The salts (3.3g) were warmed with hydrochloric acid (25 ml) and water (25 ml) until a layer of fatty acids formed on the surface of the mixture, which was thencooled and transferred to a separator. The fatty acids were extracted with light petroleum (100 ml x 3), the light petroleum extracts were bulked and washed free from lead salts and mineral acid with water. After drying the solution (Na_2So_4) the solvent was removed to give a pale yellow solid (1.6 g) m.p. 50-54°.

Esterification of saturated acids. The acids (1.6 g) were dissolved in methanol (25 ml) which contained 0.5 ml of concentrated sulphuric acid and the solution was boiled gently under reflux for two hours. The esters were isolated by diluting the solution with brine and extracting with light petroleum (100 ml x 2). The petroleum ether layers were bulked, washed successively with brine, water, saturated solution of sodium bicarbonate and water. The light petroleum layer was dried (Na_2So_4) and evaporated to give a pale yellow solid when cooled m.p. 30-35°.

The quantity of the methyl esters was too small for fractional distillation.

Saponification value[93] The ester (0.095g) accurately weighed, was dissolved in 20 ml of ethanolic

potassium hydroxide, boiled gently under reflux on a water bath for 30 minutes, cooled and the excess of alkali was neutralised with 0.1N HCl using phenolphthalein (1 ml) as indicator. A blank reading was carried out in the same way (b ml). The saponification value was calculated from the formula.

$$\text{Sap. value} = \frac{(b-a) \times 0.02805 \times 1000}{\text{wt. of ester in gm.}}$$

Saponification value of the methyl ester of the saturated acids was 230.

Iodine value. The ester (0.05-0.07g) accurately weighed was dissolved in carbon tetrachloride (5 ml) in a glass stoppered flask and a solution of iodine monochloride (20 ml) was added. After 30 minutes, potassium iodide (1g) and water (50 ml) were added and the liberated iodine was titrated with 0.1N sodium thiosulphate solution ('a' ml). A blank reading was determined in the same way ('b' ml) and the iodine value was calculated from the formula.

$$\text{Iodine value} = \frac{(b-a) \times 0.01269 \times 100}{\text{wt of ester in gm.}}$$

Iodine value of the methyl esters of the saturated acids was 4.8.

Gas chromatography of methyl esters. Gas liquid chromatography of the methyl esters was carried out on a Pye Panchromatograph under the following conditions.
Column: Apiezon L 0.5% on celite 100-120 mesh (5 ft.)
Temperature: 150°

Gas flow: 50 ml/minute.

Methyl myristate (trace), palmitate and stearate were present.

Unsaturated Acids.

Decomposition of lead salts. The filtrate from the separation of the lead salts of the saturated acids was concentrated to remove ethanol and the residue was transferred to a separator with the aid of petroleum ether (300 ml). The light petroleum layer was washed with water, dilute hydrochloric acid (to decompose any lead salts present) and again with water. The petrol was dried (Na_2SO_4) and the solvent was removed under reduced pressure to give a pale brown oily residue. (8.5 g)

Esterification of unsaturated acids. This was applied as described for saturated acids, using methanol (60 ml) and concentrated sulphuric acid (1 ml).

Fractional distillation. This was carried out using a short fractionating column. Fractions were collected at intervals and saponification value and iodine value were determined by the methods already described. The results are summarised in Table 12.

Table 12

Fraction	Colour	Bath temp. C	Dist. temp. C	Pressure m.m.Hg.	Weight (gm)	Saponification value	Iodine value.
A	colourless	240	145-147	6 m.m.	2.9	195.2	111.8
B	colourless	245	148-150	6 m.m.	4.0	194	120.8

Table 12 Continued

Fraction	Colour	Bath temp. C	Dist. temp. C	Pressure m.m.Hg.	Weight (gm)	Saponification value	Iodine value.
C	Dark brown residue	——	——	——	1.515	——	——

Identification of linoleic acid. The acid was extracted from the saponified ester of fraction (A) as a pale yellow oil which did not solidify at room temperature. It was dissolved in light petroleum (5 ml) and treated with a solution of bromine in light petroleum (2%) until bromine was in excess. The solution on cooling in a refrigerator for one hour deposited a white crystalline solid, which was filtered and recrystallised from a mixture of ether and light petroleum (1:5) as colourless needles of tetrabromostearic acid m.p. 113-115°. The crystals contained bromine and the melting point was undepressed on admixture with authentic tetrabromostearic acid. The mother liquor was concentrated under vacuum and oily crystals were obtained m.p. 30-35°.

Attempted identification of oleic acid. The acid from fraction (B) was extracted after saponification as a yellow oil which did not solidify at room temperature. A mixture of equal volumes of toluene and amyl alcohol (10 ml), one drop of phenolphthalein and a slight excess of powdered barium hydroxide (slightly more than was necessary to neutralise the oleic acid) was heated on a boiling water bath. The oily acid obtained was added to the hot suspension, and the whole shaken for a few minutes. The small amount of

solid in suspension was allowed to settle and the colourless supernatent liquid was poured off while still hot and allowed to cool, when the barium salt of the acid separated as a pale yellow solid, which was filtered, washed with toluene and recrystallised from a mixture of equal parts of amyl alcohol and toluene. The crystals were hydrolysed with a small quantity of hydrochloric acid and water to decompose the salt, but the free acid could not be identified as oleic acid.

Identification of oleic acid. The oil (0.1g) obtained from the saponification of fraction (B) was added to a mixture of hydrogen peroxide (0.1g 30%) and glacial acetic acid (0.6 ml) previously heated to 85°. The exothermic reaction was allowed to proceed, shaking occasionally and the mixture was allowed to cool slowly overnight. The solution was poured into hot water (5 ml) the aqueous layer being removed and rejected. The pale yellow oily layer was dissolved inN sodium hydroxide (3 ml) and heated for two hours on water bath. The hot solution was acidified with dilute hydrochloric acid, cooled in an ice-salt mixture, and the solidified substance removed, washed with hot water (2 ml) and recrystallised from ethanol (90%) as small glistening plates of dihydroxystearic acid m.p. 90-91°.

Gas chromatography of methyl esters. Gas liquid chromatography of the methyl esters was carried out on a Pye Panchromatograph under the following conditions.
Column: Apiezon L 0.5% on celite 100-120 mesh (5 ft.)
Temperature: 150°.

Gas flow: 50 ml/minute.

Peaks indicative of methyl oleate and linoleate were obtained.

Identification of Glycerol

Fraction (E) was neutralised with dilute solution of sodium hydroxide and evaporated to dryness under reduced pressure on a boiling water bath. The pale brown residue was mixed with anhydrous sodium sulphate (20g) and the solid was extracted by refluxing in a Soxhlet extractor with dry acetone for four hours. The acetone was cooled, filtered from salt and evaporated to give a pale brown residue (2.6g). The residue was dissolved in water, decolourised with activated charcoal, filtered and the filterate was evaporated to give a colourless residue which was dissolved in dry ethanol, filtered from a trace of salt and the filterate was evaporated to dryness. The residue was dried at 100° overnight to give a transparent liquid (2.1g) which tasted sweetish and produced warmth to the tongue and gave the following reactions.

1. Heated in a bunsen flame on a borax bead it gave a green flame.
2. Heated with a copper sulphate and sodium hydroxide solution, the liquid was coloured blue.
3. Heated with potassium bisulphite, it gave off iritating vapour of acrolein.

Glyceryl-tri-p-nitrobenzoate. The residue (0.5g) in pyridine (6 ml) was mixed with a solution of *p*-nitrobenzoyl chloride (1g) in pyridine (10 ml). The mixture was heated on boiling water bath for 30 minutes, cooled and diluted

with water (40 ml). Excess of dilute solution of sodium hydroxide was added and the mixture was placed in a refrigerator overnight to give an oily solid which was filtered and recrystallised from aqueous acetone as small glistening plate of glyceryl-tri-p-nitrobenzoate m.p. 190-192°, undepressed on admixture with an authentic sample. Ner[94] and Jaquemain and Muskovitz[95] gave m.p. 192°.

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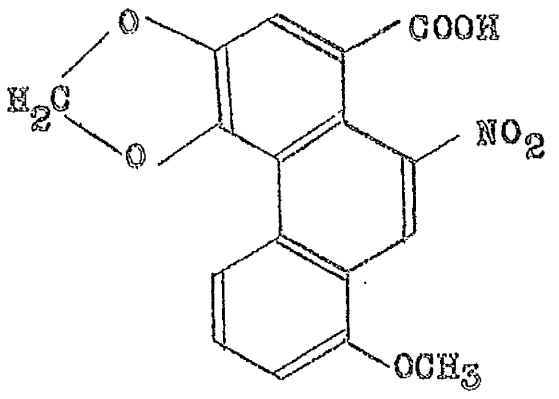
PART II

ATTEMPTED SYNTHESIS OF ARISTOLOCHIC ACIDS

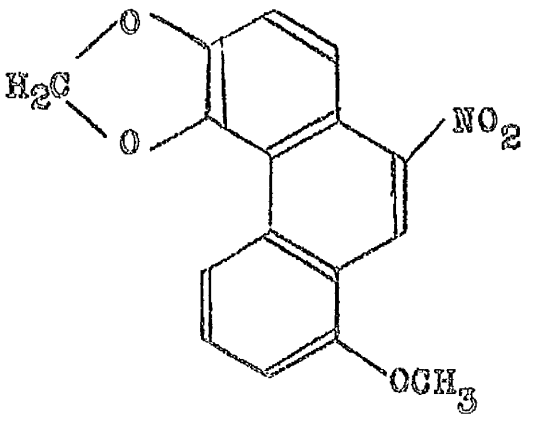
INTRODUCTION

Naturally occurring nitro compounds are few in number but Aristolochia species contain several nitrophenanthrene carboxylic acids that are of potential medicinal interest. Aristolochic acid (I) was the first of these to be isolated early in the nineteenth century probably in an impure form but it was Hesse[1] in 1895 who



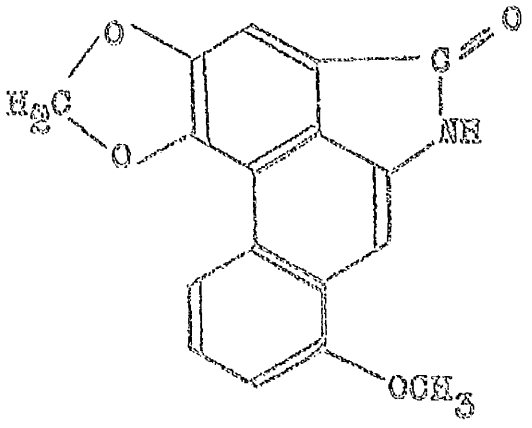
(I)

was the first to carry out a chemical examination and arrive at the correct empirical formula of $C_{17}H_{11}O_7N$. The structure was elucidated by Faller, Belohlav and Simonitsch[2,3], who isolated a mixture of acids in which aristolochic acid (I) was the main constituent from the dried powdered defatted roots and rhizomes of A. clematitis. Aristolochic acid which recrystallised from dimethylformamide-ethanol as orange-red needles $C_{17}H_{11}O_7N$ m.p. 287-292° (decomp.) formed a methyl ester $C_{18}H_{13}O_7N$, m.p. 281°. Decarboxylation of the acid with copper powder in quinoline gave the compound $C_{16}H_{11}O_5N$, (II) m.p. 216°.

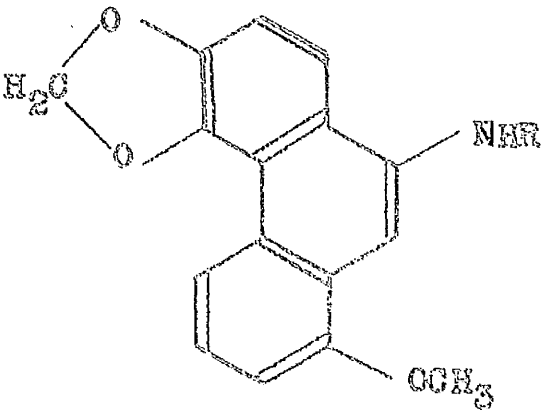


(II)

Previous workers[1,4] gave a methoxyl content of 1.5% (theoretical 9.09%) but by suitable modification of the methoxyl determination it was found that one methoxyl group was present. This was further confirmed by a similar determination on the methyl ester which was shown to contain two such groups. Zinc dust distillation of aristolochic acid gave phenanthrene which was confirmed by melting point and ultraviolet absorption. Catalytic hydrogenation of the acid and the methyl ester resulted in a hydrogen uptake of three moles with the formation of a highly fluorescent neutral compound $C_{17}H_{11}O_4N$, (III) m.p. 317-319°. The authors concluded that more than one group was concerned in this reduction and also in the reduction of the methyl ester of the acid because, with the latter, methanol was a product of the reaction. Hydrogenation of the decarboxylated acid also resulted in the hydrogen uptake of three moles to give a sensitive basic compound, $C_{16}H_{13}O_3N$, (IV, R=H) m.p. 172-173°, which was



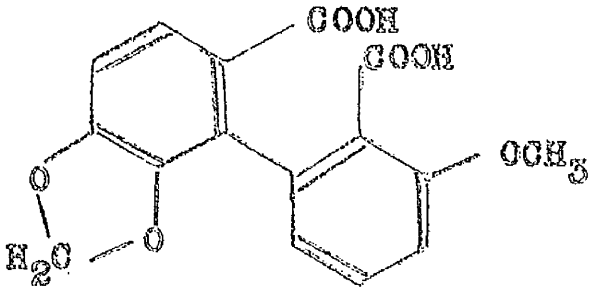
(III)



(IV)

acetylated with great difficulty. The same acetate (IV, R=Ac) was obtained by reductive acetylation of the decarboxylated compound. Diazotisation of the basic compound followed by boiling gave a nitrogen-free intensely red substance. These facts indicated the presence of a nitro group which on reduction gave the lactam of the corresponding aminoacid. Infrared and ultraviolet spectra confirmed these conclusions, which established the proximity of the nitro and carboxyl groups.

The presence of the methylenedioxy group in aristolochic acid, its ester and decarboxylated acid was shown by the liberation of formaldehyde[5] when these compounds were heated with phosphoric acid. The complete structure of aristolochic acid was established as (I) by oxidation of the decarboxylated acid (II) with hydrogen peroxide in tetrahydrofuran. The dibasicity of the resultant diphenic acid (V) $C_{16}H_{12}O_7$, m.p. 246° was confirmed by the

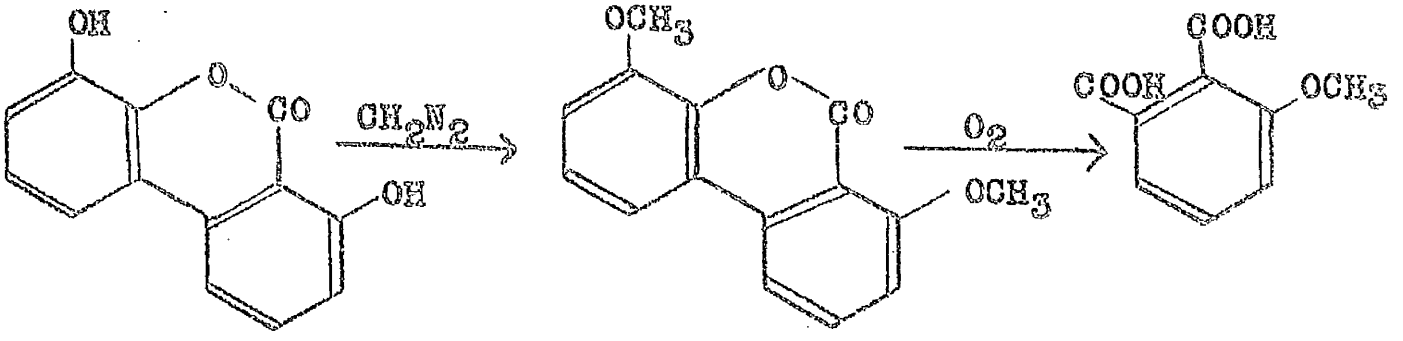


(V)

formation of the dimethyl ester $C_{18}H_{16}O_7$, m.p. 114° which was also shown to possess three methoxyl groups. The methylenedioxy group and all the carbon atoms of the starting material were still retained and from this it could be easily explained that the nitro group occupied the 9 or 10 position in the molecule. Aristolochic acid and its methyl ester gave the lactam (III) on reduction and from these observations it was quite clear that the carboxyl group must be attached to a carbon atom adjacent to C_9 or C_{10}.

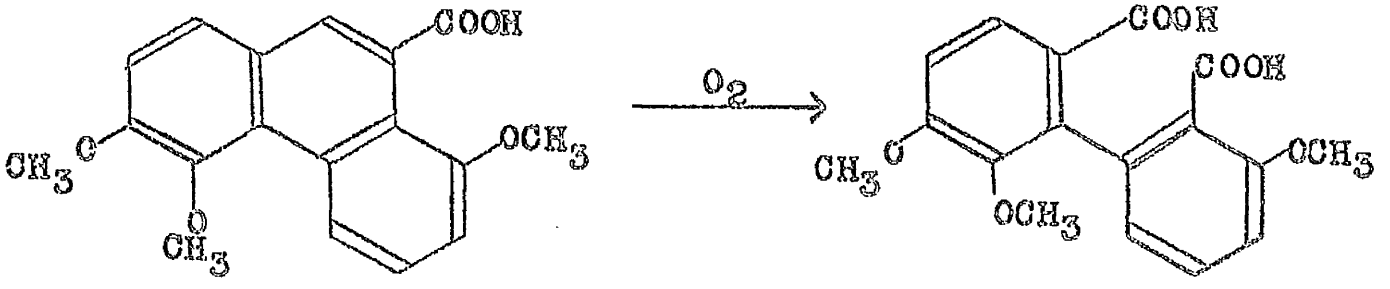
The position of the methylenedioxy and methoxyl groups was established by treatment of the diphenic acid with concentrated hydrochloric acid under pressure in the

presence of resorcinol to bind the formaldehyde released during the reaction. The other linkages were destroyed and a dihydroxylactone (VI) $C_{13}H_8O_4$, m.p. 204° was formed, the structure of which was confirmed by potassium permanganate oxidation of the corresponding dimethyl ether (VII) to O-methoxyphthalic acid (VIII) which was characterised as its anhydride. The lactone structure (VI) was



(VI) (VII) (VIII)

confirmed by synthesis of 1,5,6,-trimethoxyphenanthrene-10-carboxylic acid (IX) which was oxidised in two stages



(IX) (X)

to give a diphenic dibasic acid (X) $C_{17}H_{16}O_7$. The latter, on treatment with concentrated hydrochloric acid, gave the required lactone (VI).

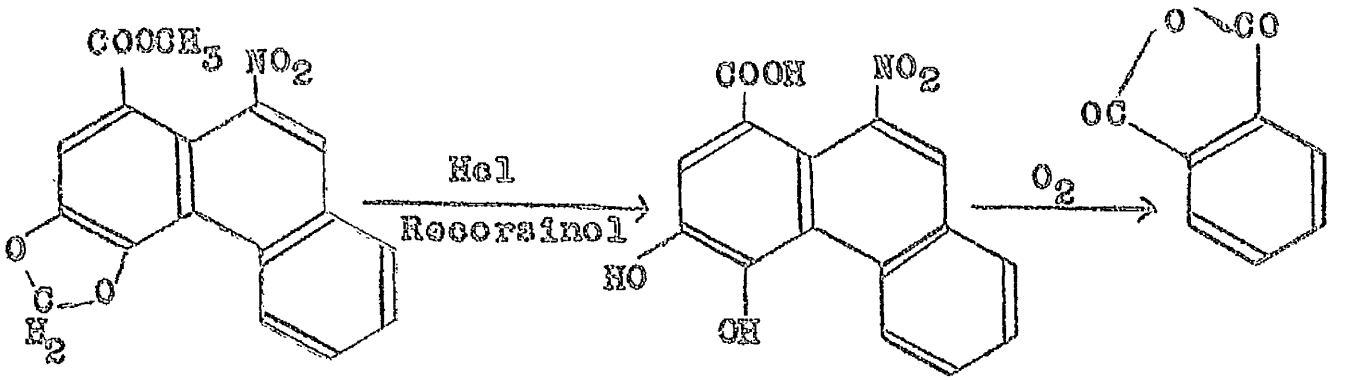
Pailer, Belohlav and Simonitsch[2,3] therefore concluded that

aristolochic acid is, 3,4-methylenedioxy-8-methoxy-10-nitro-1-phenanthroic acid. Sasagawa[6] also confirmed the structure of aristolochic acid (I) by decarboxylation of the acid to 3,4-methylenedioxy-8-methoxy-10-nitrophenanthrene(II) which was reduced with zinc and ammonium hydroxide in tetrahydrofuran to give 3,4-methylenedioxy-8-methoxy-10-aminophenanthrene isolated as the hydrochloride m.p. 230° (decomp.). This amino compound was converted to the 10-hydroxy compound by diazotisation and heating; the crude product was purified by chromatography on alumina. 3,4-Methylenedioxy-8-methoxyphenanthrene picrate $C_{16}H_{12}O_3$ $C_6H_3O_7N_3$, m.p. 174-175° was prepared and found to be identical with an authentic sample as shown by mixed melting point and infrared absorption spectra.

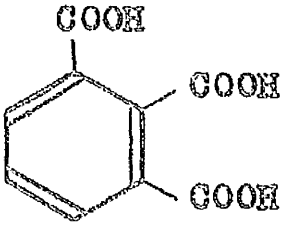
A second acid of similar structure to aristolochic acid (I) was also extracted from A. clematitis. It was first called nor-aristolochic acid[2] but in a later publication it was renamed aristolochic acid II[7]. It has the empirical formula $C_{16}H_9O_6N$, m.p. 269-271°(decomp.). Separation of aristolochic acid II from aristolochic acid (I) was difficult as fractional crystallisation of the ammonium salts and other methods such as solvent precipitation, counter current distribution and chromatography were unsatisfactory. However, separation of the methyl esters was achieved on an alumina column, the methyl ester of aristolochic acid II m.p. 274° was

eluted first followed by the methyl ester of aristolochic acid(I), m.p. 287-288°. The methyl ester of aristolochic acid II contained one methylenedioxy, one nitro, and one methoxyl group and the ultraviolet absorption spectrum showed a maximum at 215 mμ, typical of a phenanthrene derivative. It was suggested that aristolochic acid II was a methylenedioxynitrophenanthrenecarboxylic acid. Aristolochic acid II, m.p. 269-271° (decomp.), was obtained from its methyl ester in a very poor yield, so the degradative work was carried out on the methyl ester rather than the acid. Decarboxylated aristolochic acid II was easily separated from the mixture of the two decarboxylated acids by a chromatographic technique. For the structural investigation the same methods were used which were applied to elucidate the structure of aristolochic acid (I). For the same reasons, it was found that the nitro group occupied position 10 and the carboxyl group position 1 in a phenanthrene molecule.

To complete the elucidation of the structure, the methyl ester of aristolochic acid II (XI) was heated in a sealed tube with concentrated hydrochloric acid in the presence of resorcinol. The resulting dihydroxy compound (XII) was oxidised with alkaline potassium permanganate to yield phthalic anhydride (XIII) proving that the methylenedioxy group was attached to the ring containing the carboxyl group otherwise hemimellitic acid (XIV) would have been isolated. This established the

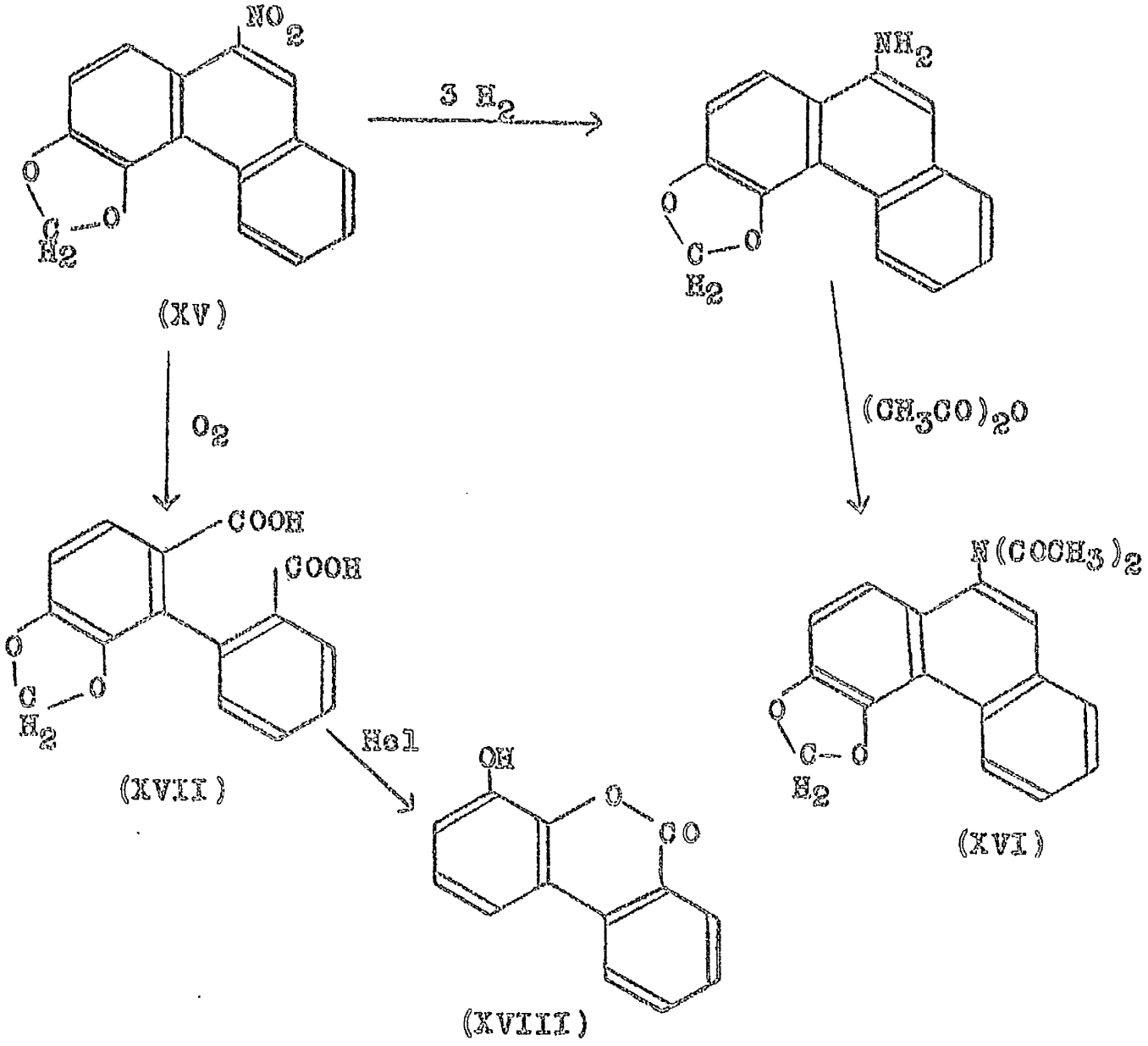


(XI) (XII) (XIII)

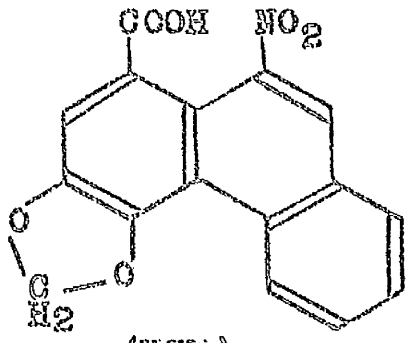


(XIV)

structure of aristolochic acid II as 3,4-methylenedioxy-10-nitrophenanthrene-1-carboxylic acid. It was confirmed by reduction of the decarboxylated aristolochic acid II (XV) followed by acetylation with difficulty to yield 3,4-methylenedioxy-10-diacetoxyaminophenanthrene (XVI) which was identical with synthetic material. The structure was further confirmed by oxidation of decarboxylated aristolochic acid II (XV) to a methylenedioxydiphenyldicarboxylic acid $C_{15}H_{10}O_6$. (XVII) which was not identical with synthetic 4,5-methylenedioxydiphenyl-2,2´-dicarboxylic acid and must therefore have been the 5,6-methylenedioxy isomer. Treatment of this isomer with concentrated hydrochloric

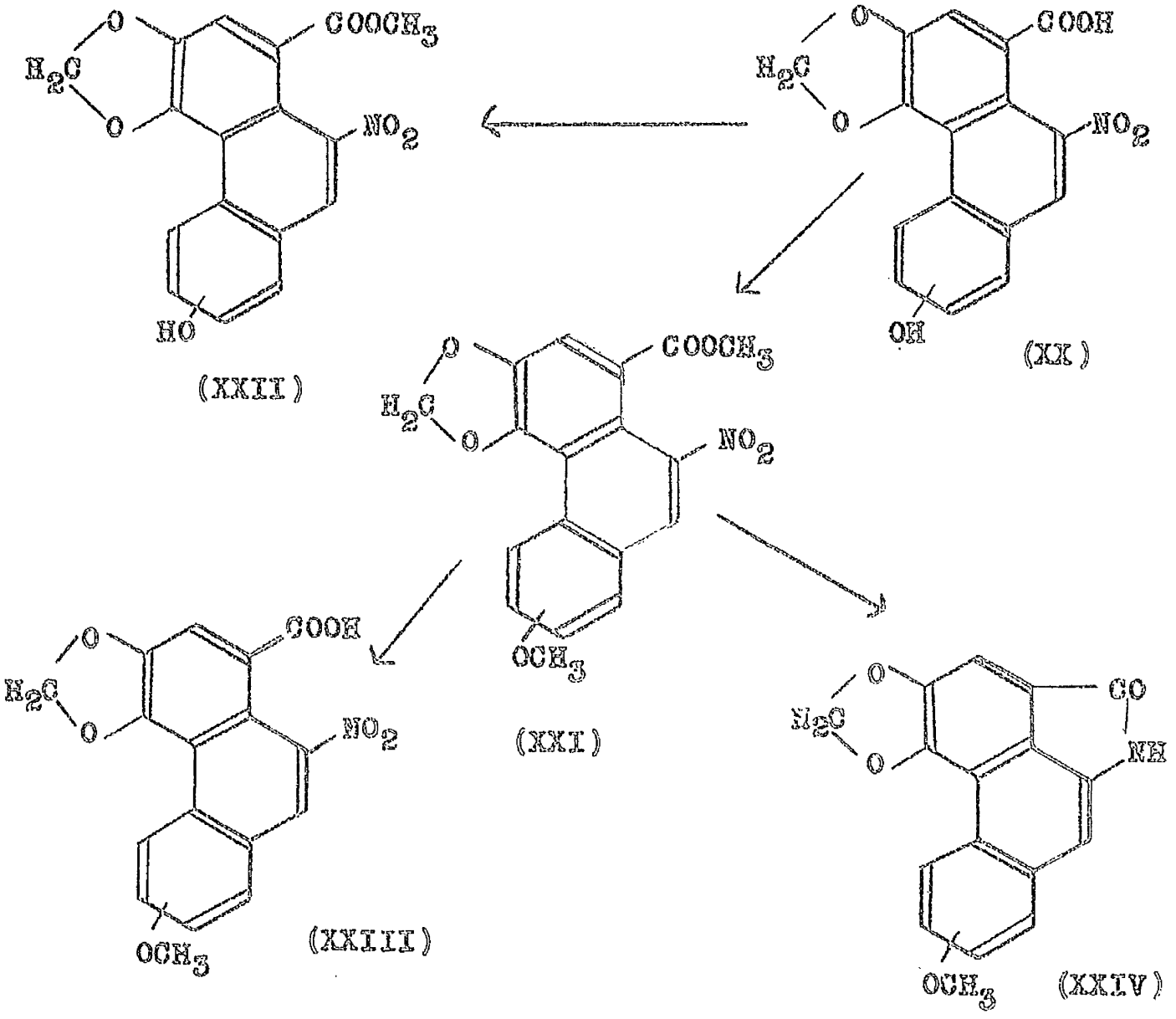


acid under pressure gave a compound identical with synthetic 3,4-benz-8-hydroxycoumarin (XVIII). Therefore aristolochic acid II is 3,4-methylenedioxy-10-nitrophenanthrene-1-carboxylic acid (XIX) and it differs from aristolochic acid (I) in not possessing a methoxyl group.



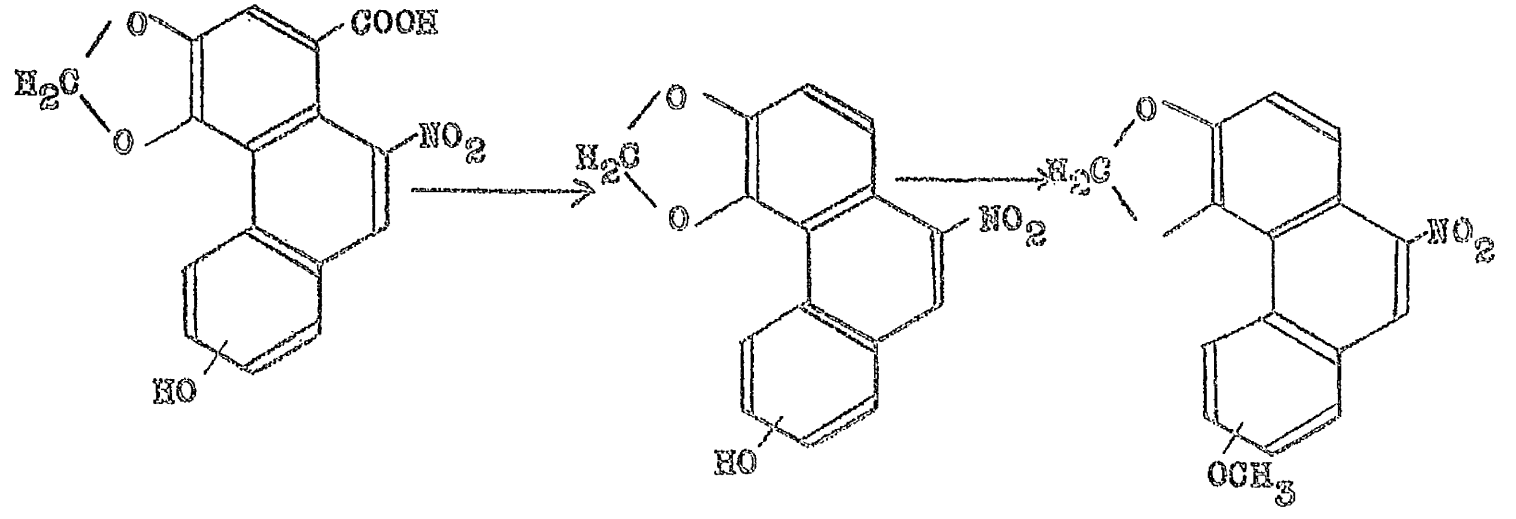
The presence of aristolochic acid (I) in A. debilis and A. kaempferi was reported by Tomita and Kura[8] who published a series of papers based on their investigation. They also isolated aristolochic acid B,[9,10] $C_{17}H_{11}O_8N$ m.p. 275-6°, methyl ester m.p. 258-60° and aristolochic acid C as yellow needles $C_{16}H_9O_7N$[6], m.p. 280° (decomp.), methyl ester m.p. 254°, from the acidic portion of the extract and from the neutral portion aristolactam $C_{17}H_{11}O_4N$, m.p. 305° was isolated. The structure of aristolochic acid C was elucidated by Sasagawa[6] who showed the presence of one nitro, one carboxylic, one methylenedioxy and one hydroxy group in a manner similar to that adopted by Pailer, Bolohlav and Simonitsch[2,3].

Methylation of aristolochic acid C (XX) with diazomethane in ether gave 1-methoxycarbonyl-3,4-methylenedioxy-x-methoxy-10-nitrophenanthrene (XXI) $C_{18}H_{13}O_7N$, m.p. 260° (decomp.). The mother liquor from the reaction yielded the methyl ester of aristolochic acid C, $C_{17}H_{11}O_7N$, (XXII), m.p. 272-5°. Hydrolysis of the O-methyl derivative of the methyl ester of acid C (XXI) with ethanolic potassium hydroxide for one hour gave x-methoxy-3,4-methylenedioxy-10-nitro-1-phenanthroic acid (XXIII) m.p. 290-5°. The O-methyl derivative of the methyl ester of the acid C (XXI) was hydrogenated with zinc and glacial acetic acid to yield the lactam (XXIV) $C_{17}H_{11}O_4N$, m.p. 230-250°.

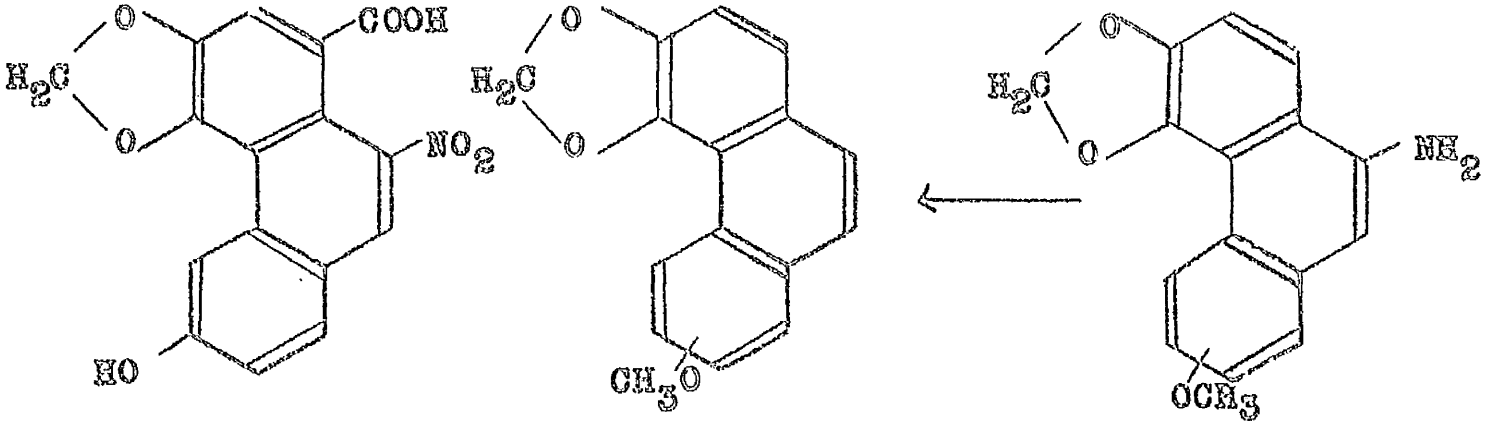


The acid C (XX) was decarboxylated by refluxing with copper and quinoline to yield a compound $C_{15}H_9O_5N$, 5 H_2O (XXV) m.p. 228° (decomp.), which was methylated with diazomethane and ether to yield the methyl ether (XXVI) $C_{16}H_{11}O_5N$, m.p. 293-5° (decomp.). Reduction with zinc and ammonium hydroxide in tetrahydrofuran gave an amino compound (XXVII) which was diazotised to yield 3,4-methylenedioxy-x-methoxyphenanthrene (XXVIII). The latter was characterised as its picrate which was

identical with authentic 3,4-methylenedioxy-6-methoxyphenanthrene picrate both in mixed melting points and infrared absorption spectra. Therefore the authors concluded that aristolochic acid C is 3,4-methylenedioxy-6-hydroxy-10-nitrophenanthrene-1-carboxylic acid (XXIX).



(XX) (XXV) (XXVI)



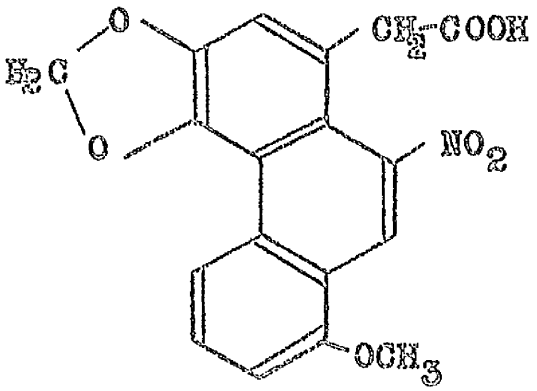
(XXIX) (XXVIII) (XXVII)

No detailed chemistry of aristolochic acid B has been reported.

Tseng and Ku[11] isolated from the roots of A. debilis yellow needles $C_{17}H_{11}O_7N$, m.p. 275° which they thought

to be isoaristolochic acid[12] but which later, was identified as aristolochic acid[13,14]. They also isolated a new nitrogen containing acid $C_{18}H_{13}O_7N$, m.p. 350° (decomp.) as yellow needles and named it debilic acid. It gave a methyl ester m.p. 260° [15] and paper chromatography indicated an R_f value of 0.87 (cf, aristolochic acid R_f 0.91-0.94). Debilic acid closely resembled aristolochic acid in chemical and physical properties. They both gave green colours with concentrated sulphuric acid and turned red in alkaline solution. Decarboxylation of debilic acid with copper and quinoline gave yellow feather like crystals $C_{16}H_{11}O_5N$, m.p. 206° which was identical with those obtained from aristolochic acid in both mixed melting point and infrared absorption spectra. Hydrogenation[16] of debilic acid with platinum oxide or with sodium mercury amalgam gave a 6-membered ring heterocyclic compound m.p. 316°, a mixed melting point with aristolochic acid lactam m.p. 319° was depressed to 290-300°. A series of homogenous crystalline compounds were obtained by mixing aristolochic acid and debilic acid which gave m.p. 260-300°. Owing to the negative result of micro oxidation of 8-methoxy-3,4-methylenedioxy-10-nitro-phenanthrene (for C-Me), the additional methylene group in debilic acid was assumed to be at position 8 and the authors suggested 8-methoxy-3,4-methylenedioxy-1-carboxymethyl-9-nitro phenanthrene as the structure of

debilic acid (XXX).



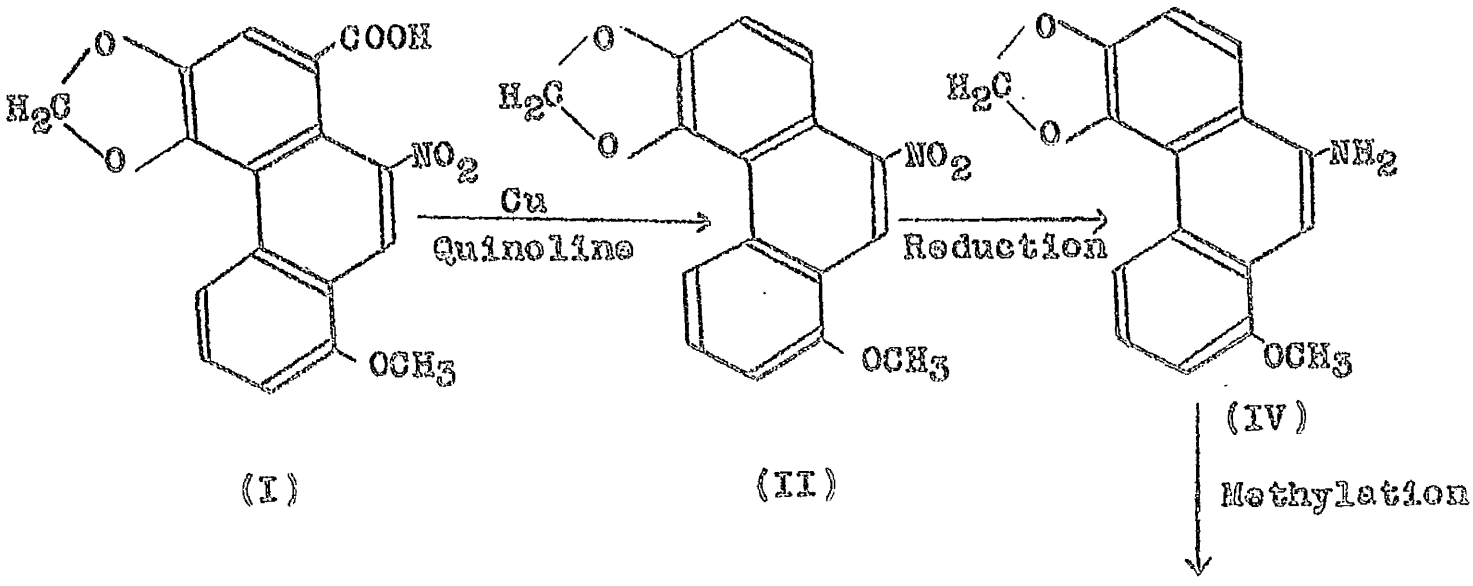
(XXX)

Tung-Tang K'O and K'uang-Fang-Tseng[17] isolated aristolochic acid $C_{17}H_{11}O_7N$, m.p. 280-1° (decomp.), from the seeds of A. debilis and they also isolated an alkaloid (reinecksate m.p. 285-6° decomp.), and red flakes $C_{20}H_{15}O_9N$, m.p. 260-1° (decomp.). It was named aristolochinic acid but no further investigation has been reported.

A non-nitrogenous acidic compound was isolated from A. cymbifera by Greene, Eugster and Karrer[18] who extracted the dried powdered roots with petroleum ether. The acidic compound, $C_{20}H_{32}O_2$, m.p. 107° was named aristolochia-cymbifera acid, but no detailed chemistry has been reported.

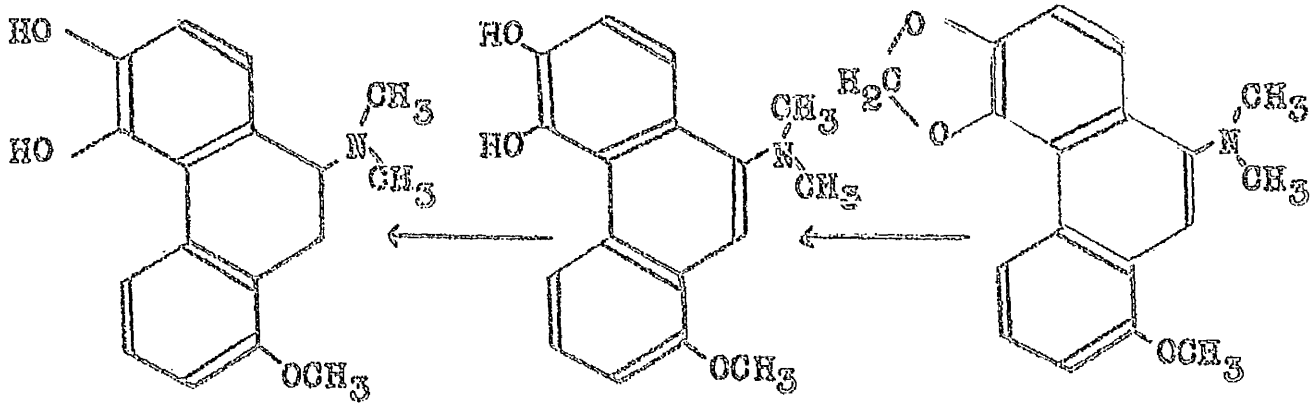
The derivatives of aristolochic acid are likely to be of considerable interest particularly in view of the reported anticancer activity of what would appear to be the lactam[19]. This has been prepared by many workers and Coutts, Stenlake and Williams[20] have shown that the so-called

diacetate of Rosenmund and Reichstein[4] is in fact the lactam acetate. The former authors also proposed a scheme[21] for the preparation of a basic substance (XXXIII) $C_{17}H_{19}O_3N$ from aristolochic acid. The basic product would be of interest because of its structural similarities to morphine (XXXIV) and apomorphine (XXXV). The amino compound 8-methoxy-3,4-methylenedioxy-10-aminophenanthrene (IV) was obtained in very poor yield by catalytic reduction of the nitro-compound. The base was remarkably unreactive to diazomethane in benzene and moist ether and also to methyl iodide in benzene. The N-methyl hydriodide was obtained in poor yield by carrying out the reaction with methyl iodide under reflux and by heating in a sealed tube. An improved yield of the base was obtained by Sasagawa[6] by refluxing the nitro-compound with zinc and ammonium hydroxide in tetrahydrofuran. The hydrochloride of the base melted at 230° and a diacetyl derivative $C_{20}H_{17}O_5N$ was also prepared. The same diacetate was obtained by reductive acetylation of the nitro-compound. The propyl and butyl esters of aristolochic acid have been prepared by Schneider[22] *via* the intermediate acid chloride, 3,4-methylenedioxy-8-methoxy-10-nitro-1-phenanthroyl chloride.



(I) (II) (IV)

Methylation



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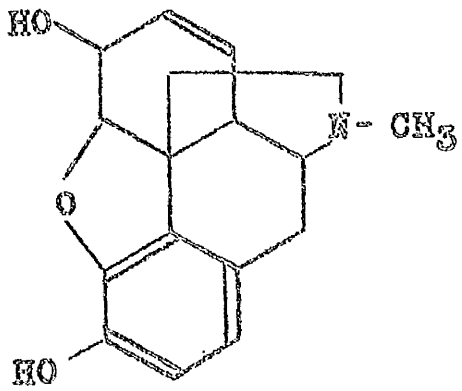
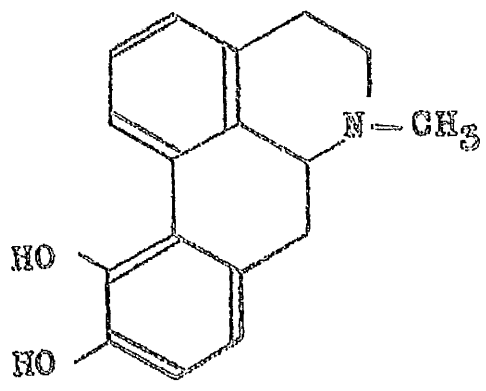


(XXXIV)



(XXXV)

The Pharmacological action of Aristolochia Species

The physiological action of Aristolochia species has aroused much interest from very early times as it was held in high esteem by the ancient Greek, Roman and Hebrew physicians on account of the reputed value in childbirth[23-26]. Extracts of the drug were also used as a bitter tonic, purgative, diuretic and in the treatment of wounds, ulcers, abscesses, fevers, asthama, epilepsy and hypertension[27]. The common name snakeroot for certain of the species is undoubtedly derived from the traditional use as a snakebite remedy. Extracts obtained from Aristolochia species have also been shown to possess C-mitotic[28] activity, to cause contraction of the uterus[29] and to inhibit cultures of Staphylococcus aureus[30], Micrococcus pyogenes[31], Micrococcus citreus, Bacillii anthracis and Bacillii subtilis[32]. Orfila[33] described Aristolochia as a narcotic poison which affected the whole nervous system. It had also been reported that extracts of A. bracteata possessed ecbolic emmenagogue[34] and abortifacient[35,36] properties due to the severe purgative action causing inflammation of the pelvic organs.

These actions of Aristolochia species, produced by extracts containing many constituents, cannot be attributed to any particular compound, but a considerable amount of work has been and is being done on the pharmacology of aristolochic acid. The first critical account is that of

Pohl[36] who found that the acid was extremely toxic to rabbits upon subcutaneous injection causing marked diurosis, paralysis and death. As is so often found, different species react differently to aristolochic acid and Pohl found that frogs and dogs were unaffected by it. A recent report of Hedwal and Peter[37] shows however, that intravenous injection of the acid into male rats induced kidney failure with a decreased glomerular filtration rate and increased blood urea and creatinine.

Recently it was found by Mose [38], Mose and Lukas[39] that aristolochic acid increased (in vitro) the phagocytic activity of leukocytes in the blood of guinea pigs. Parenteral administration of aristolochic acid gave both higher survival times and complete cures to mice which had been injected with pneumococci, but with Salmonella typhimurium it gave only slightly higher survival times but no cures. He also observed that infections or intoxications which were not influenced by leukocytes (virus infections, toxoplasmosis infection) were not affected and local treatment was less effective. After intravenous injection of the acid to rabbits an increased bactericidal action of the serum was noted. It was reported by Mehes, Decsi, Varga and Kovacs[40,41] that intravenous injection of aristolochic acid (1mg/Kg body weight) selectively caused necrosis of the epithelial cells in the proximal convoluted tubules and isochaemia of the cortical part of the rabbit kidney.

while the other part of the urinary tract remained intact. The acid did not cause renal damage in dogs but intestinal inflammation was observed and had no effect in cats, frogs and guinea pigs. Smaller doses of acid (0.1-0.2mg/Kg body weight) evoked only a partial destruction of proximal convoluted tubules. Aristolochic acid exhibits tumour inhibitory activity against adenocarcinoma in mice[42] and also inhibits malignant tumour growth both in animals and man. Although it is toxic, therapeutic doses can be administered without substantial adverse effect. A study of the patent[19] which deals with compounds and extracts isolated from A. indica reveals that more pronounced activity may lie in aristolochic acid though a compound which appears to be the corresponding lactam is also highly active. Of the crystalline compounds reported in the patent only aristolochic acid was identified. Table I records the compounds listed. Hideg, Olga, Hankovszky and Mehes[43] investigated the metabolism of aristolochic acid and its derivatives following the intravenous injection into dogs and rabbits. Aristolochic acid and its derivatives were identified in body fluid such as blood, bile and urine by paper chromatography and spectrophotometry. Aristolochic acid, in the form of its sodium salt, has also been tested against moulds[20] bacteria and yeasts but moderate activity was found only with three moulds: Penicillium notatum, Mucor sp. and Rhizopus nigricans.

T A B L E I

Compound	M.pt. °C	Analysis C	H	N %	Remarks.
Aristolochic acid	280°	59.98	3.67	4.18	
Example VI *	145	68.15	4.48	0.4	
" VIII *	230-255(Sublimed)	69.17	4.00	4.71	Green fluorescence
" XI *	269	71.99	4.59	4.4	" "
" XIII *	312	58.86	4.93	3.1	

* Patent numbers.

An alkaloid was isolated by Sokolav[44] from A. clematitis and used in the form of its hydrochloride on cats, dogs and rabbits in which animals it was found to lower the respiratory quotient. It also stimulated the respiratory centre, inhibited peristalsis, lowered muscle tonus of the womb and acted as a duiretic and cholagog.

Chang, Wang, Li, Shao, Pei, Tao Li and Hsu[45a] reported that intravenous injection of magnoflorine in cats, rats and dogs produced a prompt and significant fall of blood pressure which lasted 1 to 2 hours. Some curare-like action was also noticed. The authors concluded that the hypotensive action of magnoflorine is therefore mainly associated with the ganglionic block.

TABLE 2

SUMMARY OF *ARISTOLOCHIA* SPECIES AND CONSTITUENTS

Species	Constituent	Formula	M.P. °C	Reference
A. argentina	Aristolochine	$C_{15}H_{28}NO_3$	265	
Griseb	Palmityl Phytosterolin	$C_{42}H_{74}O_2$	82	
	Aristinic acid.	$C_{18}H_{13}NO_7$	275	1
	Aristolic acid.	$C_{15}H_{11}NO_7$	260-270	
	Aristidinic acid.	$C_{18}H_{13}NO_7$	260	
	Aristolochic acid.	$C_{17}H_{11}NO_7$	280	
A. bracteata	Aristolochic acid.	$C_{17}H_{11}NO_7$	287-292	45,46,47
Retz.	Aristored	$C_{18}H_6O_3N(OCH_3)_3$	280-2	48
	Oleic acid.			
	Myristic acid.			47
	Palmitic acid.			
	Stearic acid.			

Continued

Species	Constituent	Formula	M.P. °C	Reference
	lignoceric acid.			
	β-sitosterol			
A. clematitis L.	Magnoflorine	$C_{20}H_{24}NO_4$	248-9(iodide)	49,50
	Aristolochine	$C_{32}H_{22}NO_{13}$	215	
	Aristolochic acid.	$C_{17}H_{11}NO_7$	287-292	22,51,2
	Aristolochic acid II	$C_{16}H_9NO_6$	269-271	7.
	Sitosterol			
	Ceryl alcohol			
	Choline			49
	Trimethylamine			
	Dihydroxy Phenyl alanine			
	Caffeic acid			
	Trans-P-cumaric acid			

Continued

Species	Constituent	Formula	M.P. °C	Reference
	Sinapic acid.			
	Quinic acid.			42
	Flavanol glycerides			
A. cymbifera	Neutral compound	$C_{18}H_{22}O$	137	
Mart.	Crocetin dimethyl ester	$C_{22}H_{28}O_4$	211-212	
	Isobixin	$C_{25}H_{30}O_4$	215	18
	Aristolochia-cymbifera acid.	$C_{20}H_{32}O_2$	107	
A. grandiflora	Allantoin	$C_4H_8N_4O_3$	221	18
Gomes.	β-sitosterol	$C_{29}H_{50}O$	140	
A. debilis	Aristolochic acid.	$C_{17}H_{11}NO_7$	230	52,11,8
Sieb et Zucc.	Aristolochic acid B	$C_{17}H_{11}NO_8$	275	9,10
	Aristolochic acid C	$C_{16}H_9NO_7$	280	9,10

Continued

Species	Constituent	Formula	M.P. °C	Reference
A. debilis Sieb et Zucc	Debilic acid.	$C_{18}H_{13}NO_7$	350	12,15
	Aristolactam	$C_{17}H_{11}NO_4$	300	9,10
	Aristolochinic acid.	$C_{20}H_{15}O_9N$	260-1	17
	Alkaloid		285-6 (reineckate)	
	Magnoflorine	$C_{20}H_{24}NO_4$	249	55
	Aristolone	$C_{15}H_{22}O$		56
	Allantoin	$C_4H_6N_4O_3$	235-6	67
	Tertiary base		145	12
	Quaternary base		255 (reineckate)	
	β-sitosterol			9
	Cyclanoline		158 (picrate)	57
A. goldieana	Oleic acid			
	Linoleic acid			
	Palmitic acid			
	Stearic acid			
	β-sitosterol			

Continued

Species	Constituent	Formula	M.P. °C	Reference
A. indica	Aristolochine	$C_{17}H_{19}NO_3$	215	
	Isoaristolochic acid.	$C_{17}H_{11}NO_7$	275	
	Phytosterolin			58
	Ishwarone	$C_{15}H_{24}$		
	Ishwarone	$C_{15}H_{22}O$		
	Ishwarol	$C_{15}H_{23}(OH)$		
	Allantoin	$C_4H_8N_4O_3$		
	Oleic acid			
	Linoleic acid			
	Palmitic acid			
	Stearic acid			58
	Lignoceric acid			
	Cerotic acid			
	Glycerol			
	Ceryl alcohol			

Continued

Species	Constituent	Formula	M.P °C	Reference
	Phytosterol		137-8	
	Aristolochic acid	$C_{17}H_{11}NO_7$	290	59
A. kaempferi Willd	Magnoflorine	$C_{20}H_{24}NO_4$		
	Aristolochic acid	$C_{17}H_{11}NO_7$	290	8
A. longa L.	Aristolochic acid	$C_{17}H_{11}NO_7$	290	59
A. Maxima Jacq.	Aristolochic acid	$C_{17}H_{11}NO_7$	290	60
A. pandurata Jacq.	Aristolochic acid	$C_{17}H_{11}NO_7$	290	60
A. reticulata L.	Terpene	$C_{10}H_{16}$		61
	Acetic acid			62
	Maleic acid			

Continued

Species	Constituent	Formula	M.P. °C	Reference
A. reticulata L	Oxalic acid			
	Aristolochine			
	Glucose			62
	Water-insoluble acid	$(C_5H_8O_2)_n$	72	
	Borneol	$C_{10}H_{18}O$		
	Δ^4 Carene	$C_{10}H_{16}$		68
	Aristolactone	$C_{15}H_{20}O_2$	111	
	Quaternary alkaloid	$C_{17}H_{20}NO_3Cl$		
	Iso-rhamnetin	$C_{16}H_{12}O_7$	318-322	59
	Aristolochic acid	$C_{17}H_{11}NO_7$	280	
	Aristo-red.	$C_{19}H_{15}NO_6$		
	Allantoin	$C_4H_8N_4O_3$	221	
	β-sitosterol-β-D-glucoside	$C_{35}H_{60}O_6$		21
	Reticulene	$C_{15}H_{24}$		

Continued

Species	Constituent	Formula	M.P. °C	Reference
A. rotunda	Aristolochine	$C_{32}H_{22}N_2O_{13}$	215	63
A. serpentaria	Borneol	$C_{10}H_{18}O$		64
L	β-sitosterol	$C_{29}H_{50}O$	140	65
	β-sitosteryl-β-D-glucoside	$C_{35}H_{60}O_6$		21, 65
	Aristolochic acid	$C_{17}H_{11}NO_7$		59, 60
	Aristored	$C_{19}H_{15}NO_6$		21.
	Aristolactone	$C_{15}H_{20}O_2$	111	21
A. siphb L'Herit	Aristolochic acid	$C_{17}H_{11}NO_7$	280	53
A. Zenkeri	d-Borneol	$C_{10}H_{18}O$		
	l-Camphene			
	Oil			
	Butyric acid.			

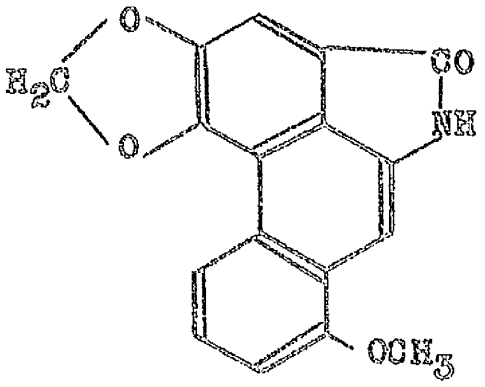
Continued

Species	Constituent	Formula	M.P. °C	Reference
A. Zenkeri	Palmitic acid			54, 66
	γ-crocetin			
	Steroid Compound	$C_{18}H_{18}D_5$	130-0.5	
	Ceryl alcohol			
	β-sitosterol			
	α and β Carotene			
	Resin (1)			
	Resin (11)			
	Dextrose			
	Levulose			
	Sucrose			
	Starch			
	Cellulose			
	Pectins			

D I S C U S S I O N

Derivatives of Aristolochic acid

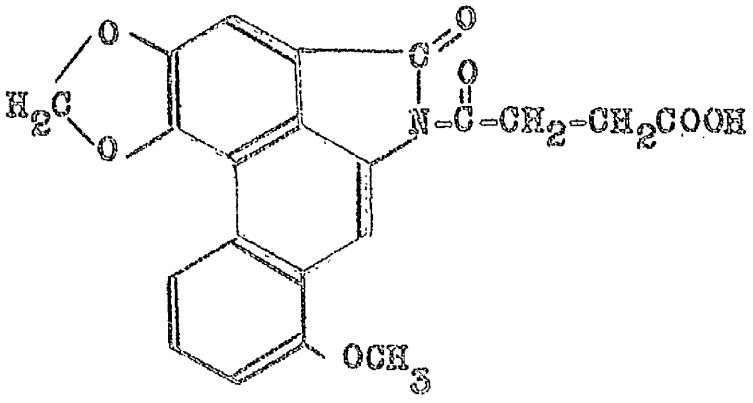
The filing of U.S. patent 895037 by Chas. Pfizer[19] & Co. Inc., covering substances in Aristolochia species that inhibited the growth of tumours, stimulated the research work already in progress in these laboratories. A quantity of powdered root of A. indica and A. longa was available and aristolochic acid was isolated therefrom with the first object of preparing derivatives from the lactam (III). The isolation procedure



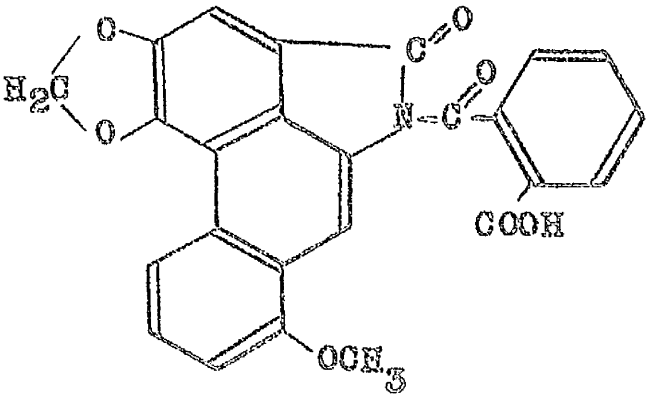
(III)

followed the normal pattern adopted by Coutts[21] and the lactam and its acetate were prepared without undue difficulty. Three compounds were thus available for testing. A fourth and new compound the lactam benzoate was obtained by the action of benzoyl chloride on the lactam in the presence of pyridine. As the lactam and its derivatives were insoluble or only slightly soluble in many solvents, attempts were made to introduce the carboxylic acid group, as a solubilising group into the

lactam. It was hoped to accomplish this by treatment of the lactam with succinic and phthalic anhydrides to give compounds (XXXVI) and (XXXVII). The attempts, however



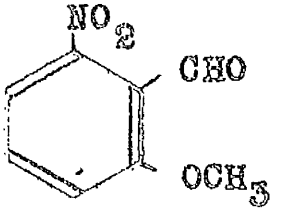
(XXXVI)



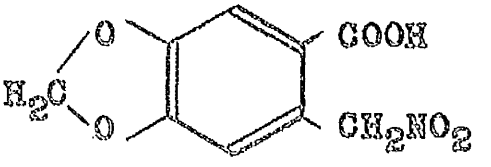
(XXXVII)

were unsuccessful in spite of a variety of experimental conditions as reported in Table 3. As Coutts[21] had experienced considerable difficulty in the isolation of an amine derivative and Japanese workers[6] had forestalled our proposed route to obtain better yields by a method involving reduction with zinc under alkaline conditions it was decided to break off this aspect of the work. Since the elucidation of the structure of aristolochic acid by Pailer Belohlav and Simonitsch[2,3] the presence of aristolochic acid has been reported in more Aristolochia species (Table 2) but no attempts have been made to synthesise the acid. This omission prompted the work reported in the following pages.

There are several syntheses of the phenanthrene nucleus and among them that of Pschorr[69,70,71] appeared to be worthy of first choice because, of the required intermediates (XXXVIII & XXXIX), one of them (XXXVIII) had already been prepared by Ashley, Perkin and Robinson[72] and by Shirai and Oda[73]. Moreover, little

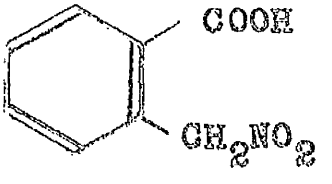


(XXXVIII)



(XXXIX)

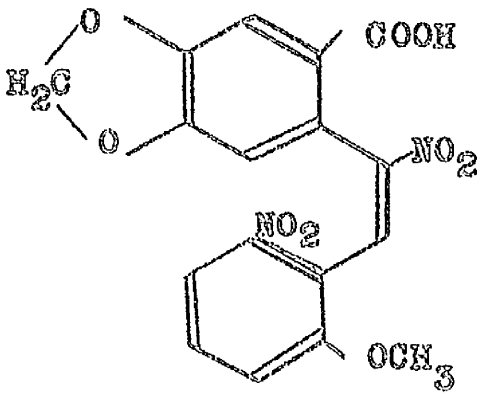
difficulty was expected in the synthesis of fragment (XXXIX) as a somewhat similar compound (XL) had been prepared by Pailer, Woerther and Meller[74].



(XL)

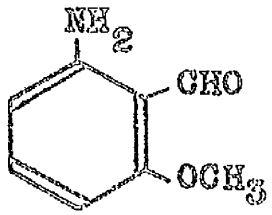
In the Pschorr synthesis the condensation of the aldehyde portion with the other reactant is achieved because of the reactive methylene group on the latter. Usually this is due to the activating influence of the carboxyl group, as for example, in phenylacetic acid but it was expected that a nitro group would also be satisfactory; particularly so

because it is known that o-nitrotoluene[75] and phenylnitromethane[76] will condense with the appropriate aldehydes. The expected product would therefore be (XLI). Clearly, a difficulty



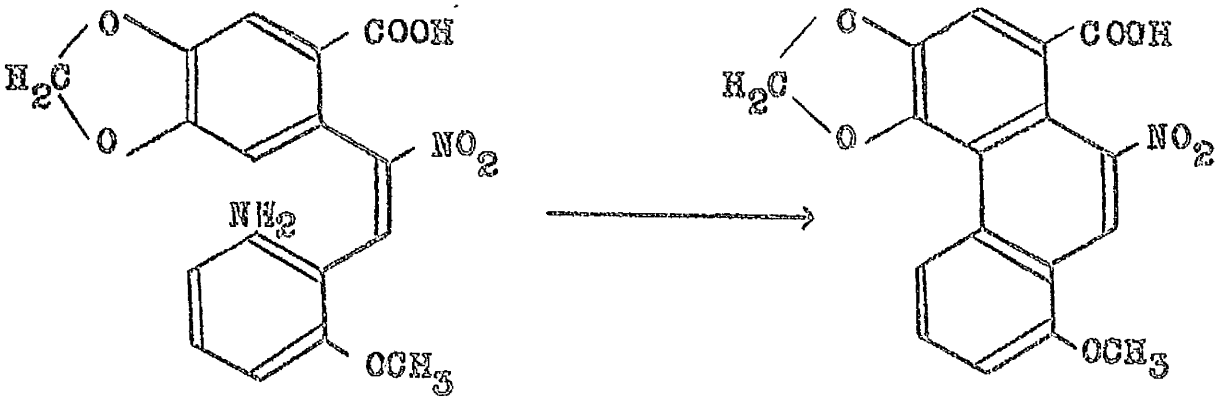
(XLI)

would arise in the next stage when the aromatic nitro group only must be reduced. If this selective reduction should prove impossible to achieve then condensation with the intermediate (XLII) would be required



(XLII)

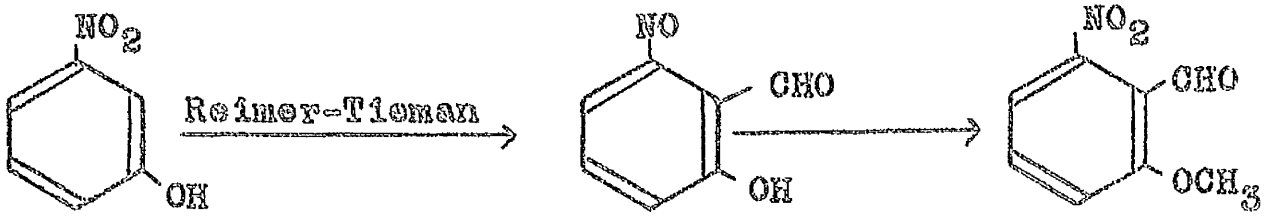
or possibly its acetyl derivative as the aminobenzaldehydes are unstable. Diazotization and ring closure should then give aristolochic acid.



(XLIII) (I)

2-Methoxy-6-nitrobenzaldehyde.

This compound was prepared in low yield (5%) by using the method adopted by Ashley, Perkin and Robinson[72] and Shirai and Oda[73]. No difficulty was experienced in

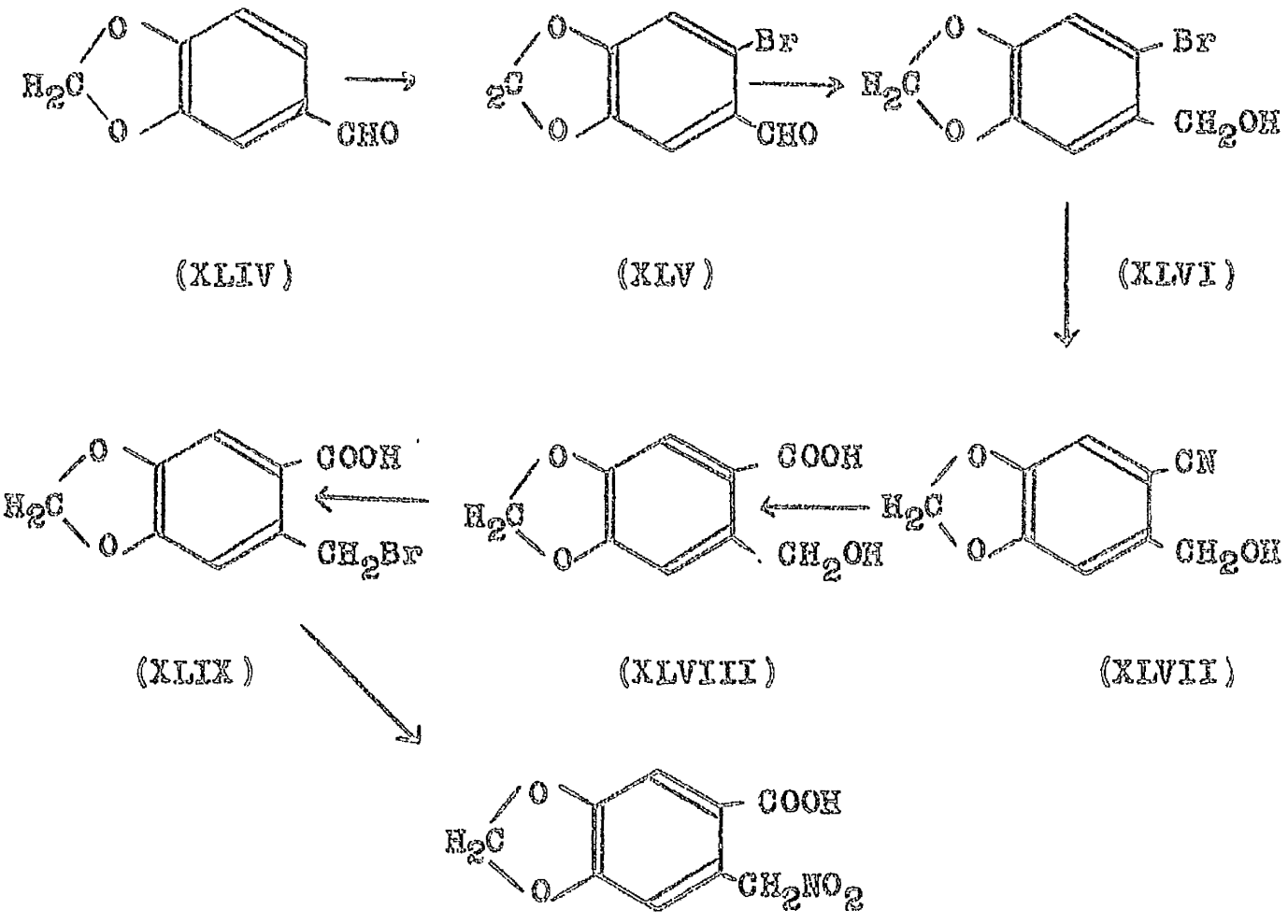


(XXXVIII)

this, the low yield being due to the Reimer-Tieman stage of the synthesis. This is well known and in latter stages of this work many trial condensation experiments were made with readily available o-nitrobenzaldehyde and benzaldehyde so as to conserve material.

6-Carboxy-3,4-methylenedioxyphenylnitromethane

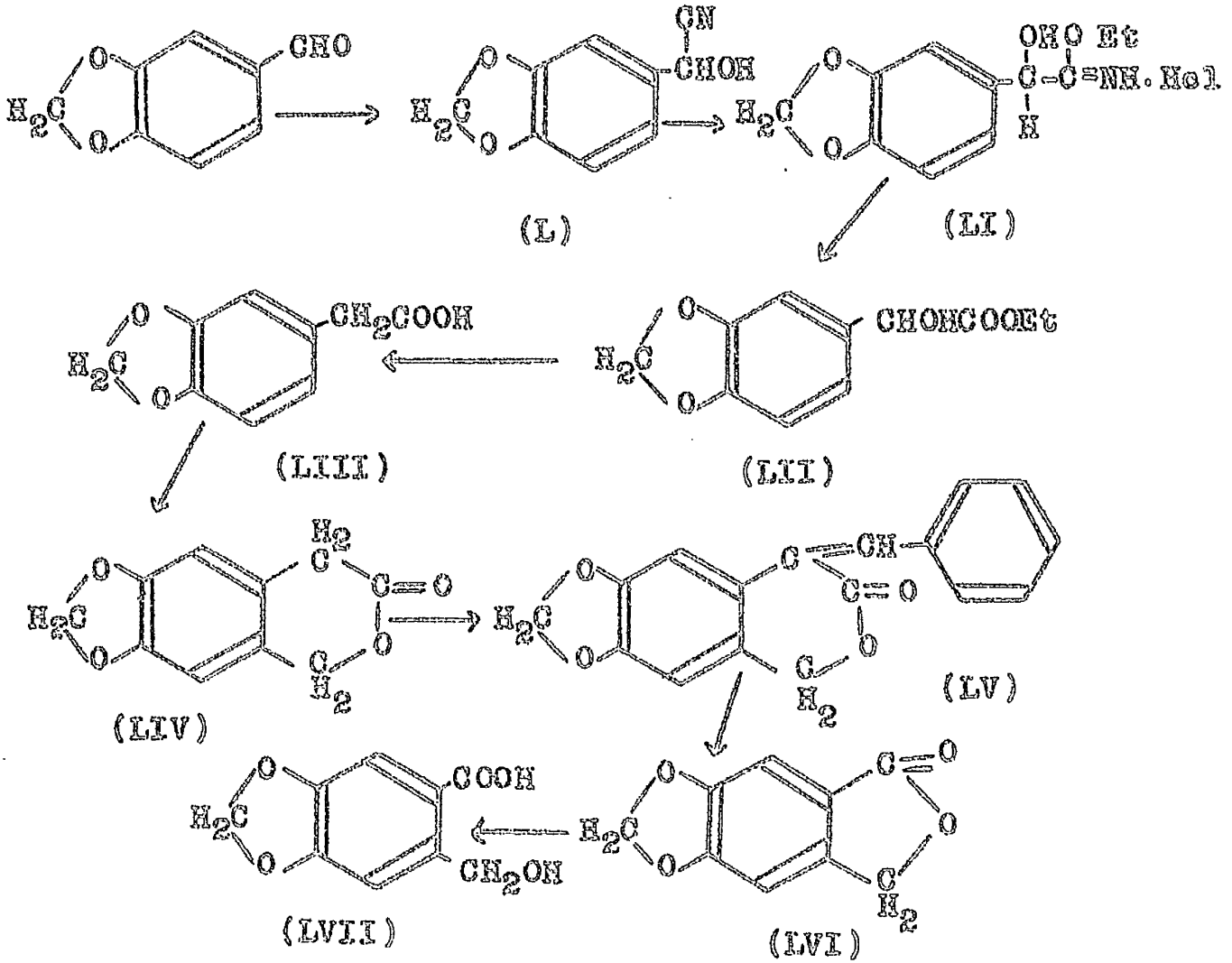
An obvious starting material for the synthesis of the nitro-acid (XXXIX) was piperonaldehyde (XLIV), and the proposed route was as follows:



Bromination of piperonaldehyde by the method of Orr, Robinson and Williams[77] gave 6-bromopiperonaldehyde (XLV) in good yield. Naik and Wheeler[78] obtained the corresponding alcohol, by reduction with lithium aluminium hydride, and using their method the alcohol (XLVI) was prepared in 95%

yield. Conversion of the aromatic halogen compound to the cyano compound was attempted both at high temperatures (170° and 205°) and at lower temperature (refluxing pyridine and 145°). Under the latter conditions starting material was isolated but above 170° decomposition occurred. All attempts to prepare the cyano-compound (XLVII) therefore proved fruitless and this route had to be abandoned.

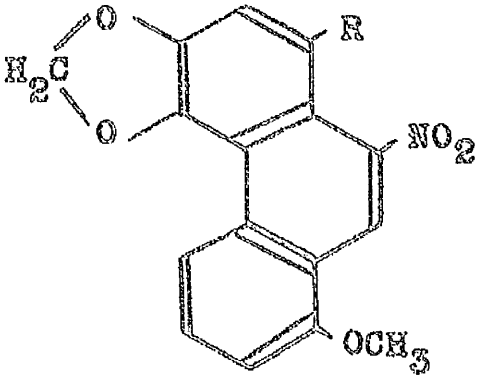
The rather longer route, to 3,4-methylenedioxy-6-hydroxymethylbenzoic acid (XLVIII) shown in the following scheme was therefore adopted.



This route is a combination of the work of Barger and Ewins[79], Stevens[80] and Stevens and Robertson[81]. The latter noted that whereas the hydroxy acid liberated from compound (LIV) lactonised spontaneously, that from 4,5-methylenedioxyphthalide (LVI) was comparatively stable. However, a disquieting feature observed by Stevens[80] was the remarkable mobility of the halogen in methylenedioxybenzyl halides and this undoubtedly had a bearing on the next step in the route viz the attempted preparation of 6-bromomethyl piperonylic acid (XLIX). Hydrogen bromide under ethanolic and aqueous conditions yielded the lactone (LVI), a result not unexpected. All attempts to protect the carboxyl group by ester formation via the silver salt and by treatment with diazomethane proved fruitless, and the lactone separated in every experiment. Similarly the reaction with phosphorous pentachloride and with thionyl chloride yielded the lactone rather than the expected 6-chloromethyl-3,4-methylenedioxybenzoylchloride. In the absence of the methylenedioxy group the reaction proceeded smoothly in agreement with Clarke[82] and the difficulty encountered must therefore be due to the influence of the methylenedioxy group.

3,4-Methylenedioxyphenylnitromethane

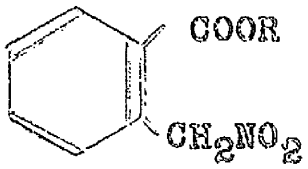
In view of the lack of success with this carboxy compound simpler products such as 3,4-methylenedioxy phenylnitromethane and its 6-bromo-compound were considered as possible intermediates in the preparation of the decarboxylated aristolochic acid (LVII, R=H) and the corresponding bromo-compound (LVII, R=Br). The latter could conceivably be converted to aristolochic acid by replacement of bromine by the nitrile group or by a Grignard reaction, though the failure to introduce the nitrile group earlier made this rather doubtful.



(LVII)

Aliphatic nitro-compounds are generally prepared by the action of silver[83,84] or sodium nitrite[85] on halogen compounds. Silver nitrite is the reagent of choice[86] for compounds containing the primary halogen group but a mixture in various proportions of nitro-compound and nitrite is often obtained depending upon the conditions of

the reaction and on the nature of both the halogen and the alkyl group[87]. Homopiperonyl bromide and 6-bromohomopiperonyl bromide were therefore prepared in good yields from piperonaldehyde via the corresponding homopiperonyl alcohols by reactions reported in the literature. The first reaction tried with homopiperonyl bromide and silver nitrite was based on the method of Pailer, Woerther and Meller[74] for the synthesis of the compounds (LVIII, R=C_6H_5 and C_2H_5).



(LVIII)

The relative ease of preparation of these reported compounds influenced the choice of route actually adopted (Page 143). No undue difficulty appeared to the German workers but considerable decomposition arose in our hands using the corresponding 3,4-methylenedioxy compounds and the various conditions shown in Table 4 (Page 147). The reaction between metal nitrite and primary halide is usually carried out at room temperature[88], but occasionally at various higher temperatures[89]. Under these conditions however benzyl halide is converted to benzaldehyde[90,91] through the intermediate nitrite or to

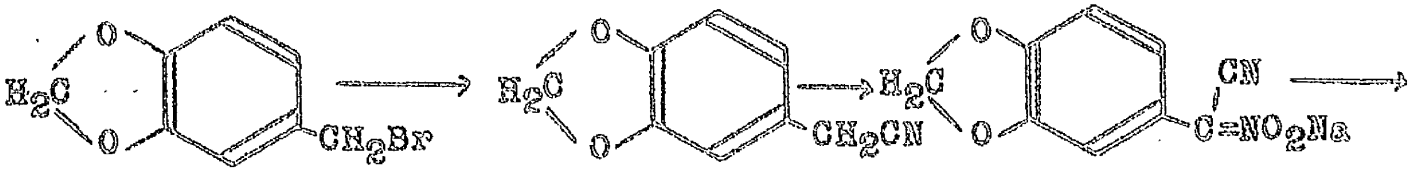
benzoic acid[92,93] through the formation of the nitrolic acid — reactions (1) and (2).

(1) $2\,RCH_2ONO \longrightarrow RCH_2OH + RCHO + 2NO$ [94-97] $(R=C_6H_5)$

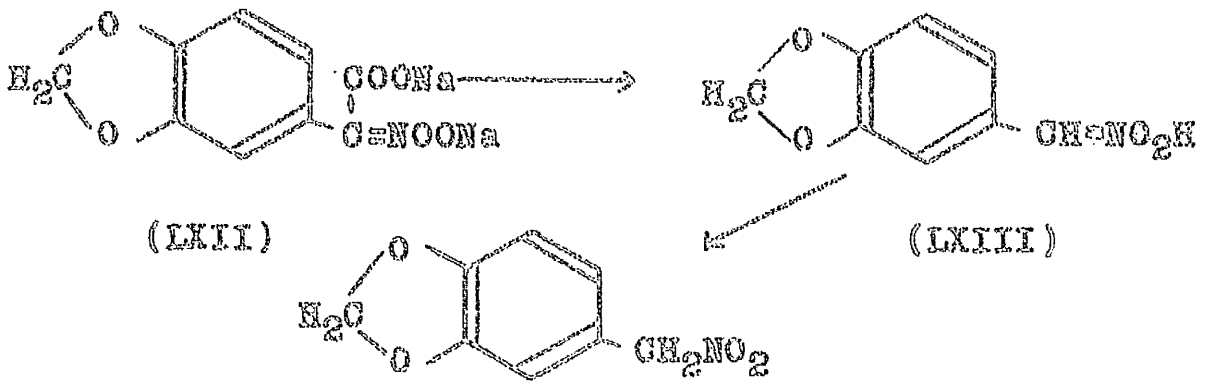
(2) $RC(NO_2)=NOH \longrightarrow RCOOH \quad (R=C_6H_5)$

Silver nitrite and homopiperonyl bromide both in dry conditions as well as in various solvents (Table 4) at room temperature either gave rise to piperonaldehyde or no reaction occurred. The origin of the aldehyde is readily explained on the basis of equation (1) above $(R=C_6H_3O_2CH_2)$.

In view of these results the little used method for phenylnitromethane reported in Organic Syntheses[98] was tried with immediate success using homopiperonyl nitrile. The reaction sequence is.



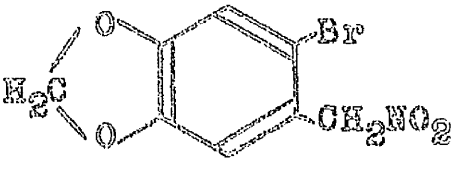
(LIX) (LX) (LXI)



(LXII) (LXIII)

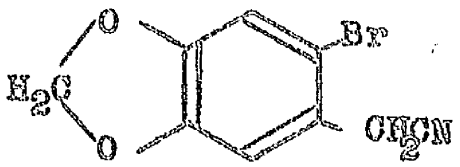
(LXIV)

3,4-Methylenedioxyphenylnitromethane was isolated as pale yellow needles m.p. 55°. It was also prepared from sodium nitrite and homopiperonyl bromide according to the method of Kornblum, Larson, Blackwood, Mooberry, Oliveto and Graham[92] for the preparation of phenylnitromethane. It was identical in all respects with the compound isolated <u>via</u> the nitrile but the yield was 30% as compared with 70%. The corresponding 6-bromo-compound (LXV) was also obtained in 75% yield by the nitrile method as well as by the action of sodium nitrite on the corresponding bromo compound.



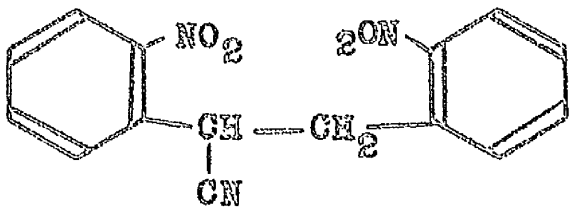
(LXV)

An interesting side reaction occurred during the preparation of the intermediate nitrile (LXVI) required for the above reaction.



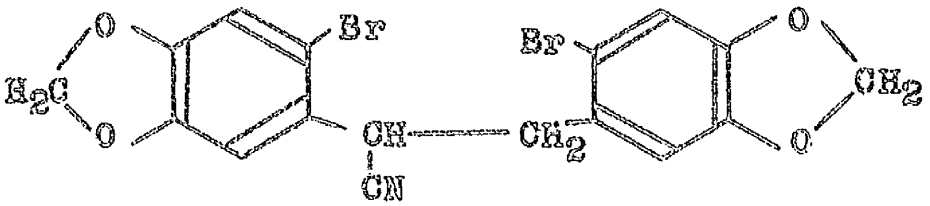
(LXVI)

White shining flakes, m.p. 184-185° were obtained and these showed nitrile absorption at 2225cm^{-1}. By analogy with the reaction of potassium cyanide on o-nitrobenzyl chloride leading to o-dinitrocyanodibenzyl[99] (LXVII) it was expected that this compound was also the corresponding nitrile (LXVIII).



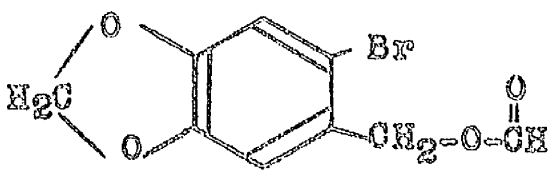
(LXVII)

The analytical figures for C, H, N and Br and molecular weight are in complete agreement with this formulation which is also supported by the infrared absorption curve of the compound. This bears some resemblance to that of the starting material as would be expected.



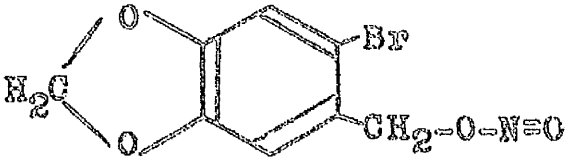
(LXVIII)

Experiments carried out with 6-bromohomopiperonyl bromide at the same time as those discussed under homopiperonyl bromide yielded results of some interest. Contrary to literature reports of the effectiveness of the silver nitrite technique it failed to yield the desired nitro-compound and gave mainly 6-bromopiperonaldehyde as detailed in Table 5 (page 152). These results are similar to those in table 4 (page 147) for homopiperonyl bromide. However, further experiments carried out in dimethylformamide for 15 minutes at room temperature led to the formation of a nicely crystalline compound m.p. 93 -95° which contained no nitrogen. The infrared absorption curve showed ester absorption at 1705cm $^{-1}$ and elemental analysis indicated C, 42.7, H, 2.67%. Although the formate ester (LXIX) requires C, 41.7% it appeared worthwhile to prepare authentic ester by dissolving silver nitrite in formic acid and adding the solution to the bromohomopiperonyl bromide in dimethylformamide.



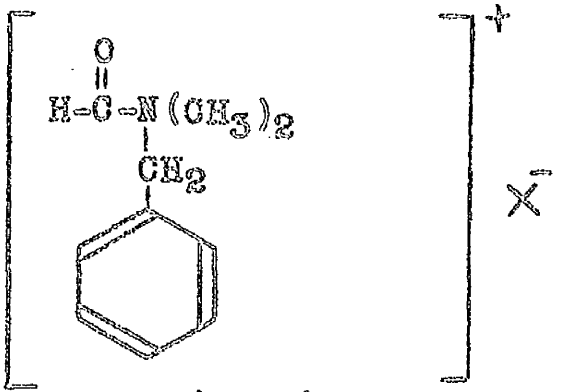
(LXIX)

The product was identical in all respects with that first isolated. Because of the complications[100] that might arise by operating at room temperature the reaction was carried out at -15 to -20 for 6 hours and again the formate ester was isolated. Free formic acid was absent from the dimethylformamide, which was freshly distilled, and the course of the reaction is a matter of some conjecture. To account for ester formation it would appear that an intermediate must be the nitrite ester (LXX) in order that the -C-O- bond be formed.



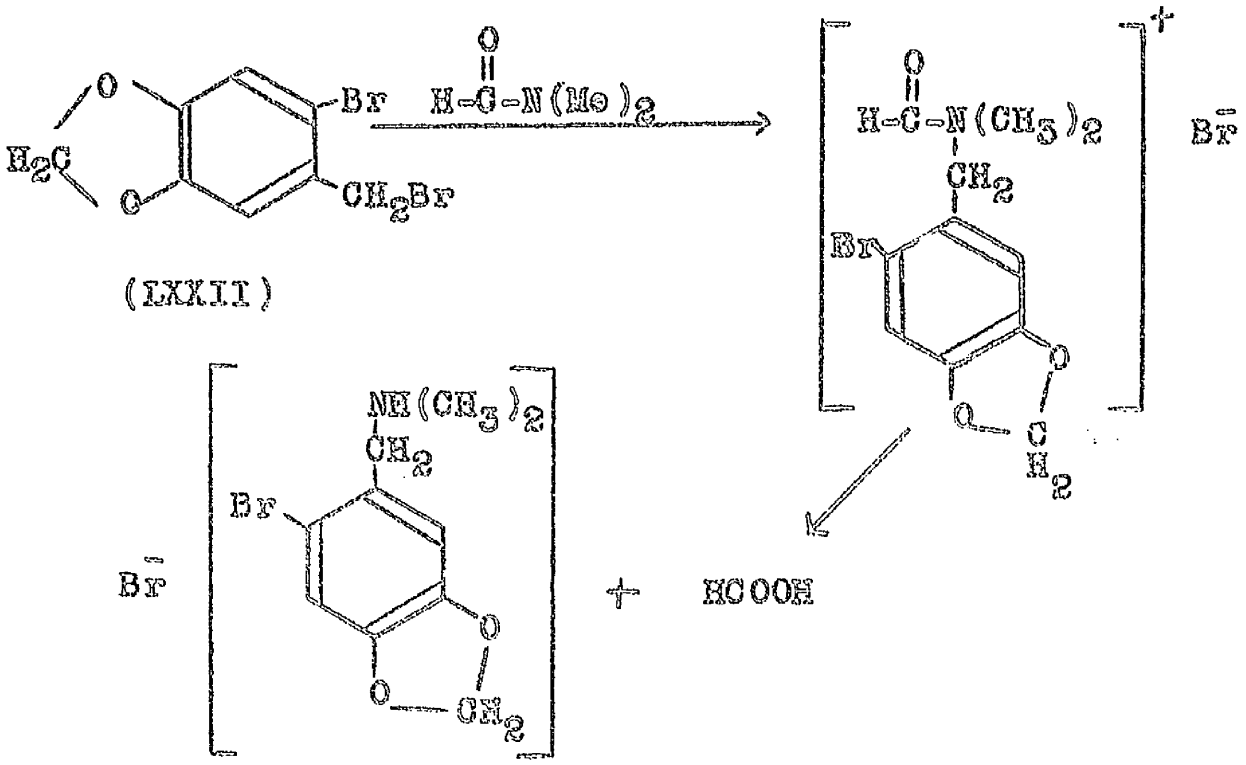
(LXX)

An alternative explanation is based on the work of Kornblum and Blackwood[101] who have shown that dehydrohalogenation of benzylbromide takes place in dimethylformamide, presumably to form a salt (LXXI) which hydrolyses to give formic acid.



(LXXI)

The origin of the formate ester may therefore be as follows.



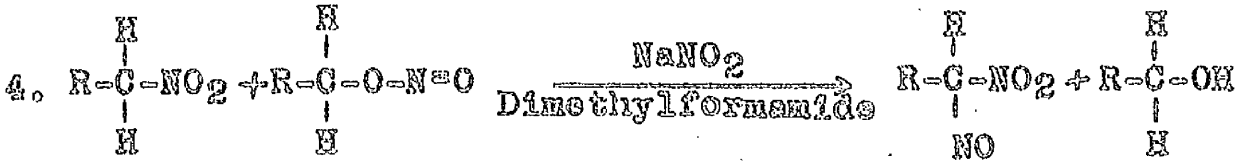
(LXXII)

The formic acid produced, in the presence of silver nitrite, reacts with more bromo-compound (LXXII) to yield the formate ester in the normal way.

There is, however, an objection to this scheme in that the rate of formation of the intermediate "Salt" is slow. The authors state that the reaction of benzyl bromide in the dimethylformamide is 10% complete in 19 days. The reaction with silver nitrite is instantaneous as shown by the immediate precipitation of silver bromide, and addition of water to the mixture precipitates the ester. Of the two schemes, therefore, nitrite formation is more likely.

As expected when glacial acetic acid was used as solvent, although no nitrous fumes were evolved on adding the silver nitrite, the acetate ester was isolated. This was identical with an authentic sample prepared by the method of Barthell and Alexander[102]. Although sodium nitrite is reported to be of less value than silver nitrite for the preparation of nitro-compounds from primary bromides, the method of Kornblum, Larson, Blackwood, Hooberry, Oliveto and Graham[92] actually yielded the required nitro-compound. It was identical in all respect with the compound isolated *via* the nitrile synthesis but was obtained in approximately half the yield. The isolation of the nitro-compound is contrary to the general opinion[103-106] that the reaction of alkyl halides with alkali metal nitrite produces nitrite ester rather than nitro-compound. However it has been shown that the reaction of sodium nitrite with alkyl halide is a simple and effective way of obtaining pure nitro-compounds provided appreciable amounts of both alkali metal nitrite and the alkyl halides are in solution, otherwise the reaction does not take place. For this purpose dimethylformamide is the solvent of choice for it not only has the requisite solvent properties but in addition the reaction in this medium is exceptionally fast[107]. Because of the speed of reaction the processes which destroy

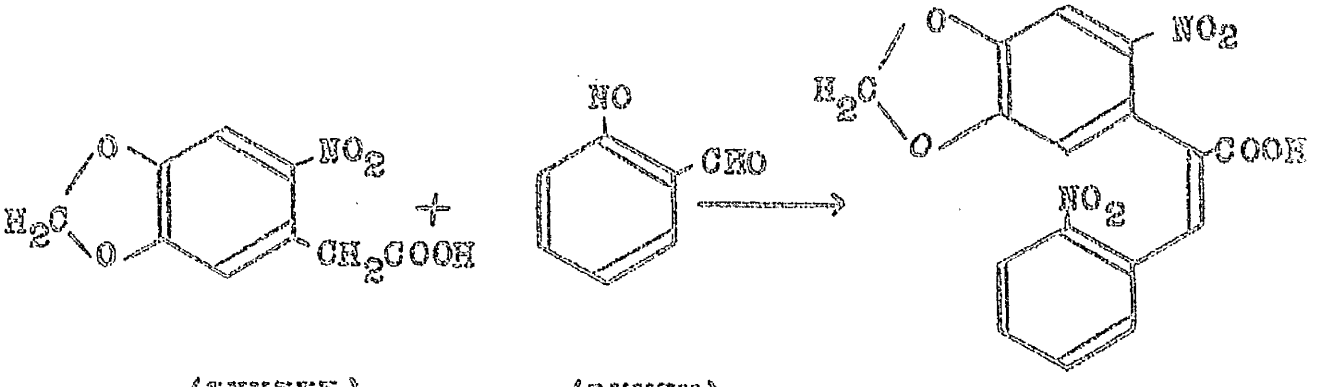
the initially produced nitroparaffins and alkyl nitrite do not have the chance to intrude and it is possible to minimise the side reaction[107] which is as follows:-



Kornblum and Weaver[93] used urea to increase the solubility of sodium nitrite in dimethylformamide and they also used dimethylsulfoxide as a solvent. Kornblum, Blackwood and Powers[108] mentioned in their work that the reaction of primary bromide with sodium nitrite at room temperature is so much faster than the competing process, that merely working up the reaction mixture promptly prevents intrusion by the reaction equation (4) . They used phloroglucinol as a scavenger particularly for the preparation of nitro-ester. It reacts with nitrite esters to form a deeply coloured material presumably nitrophloroglucinol which is readily soluble in water and non-volatile, thus facilitating the isolation of pure colourless nitro-compounds. In our own work however, phloroglucinol was not used.

Condensation Reactions

In order to gain experience in the condensation of substituted benzaldehydes with active methylene groups, some experiments were carried out with 6-nitrohomopiperonylic acid (LXXIII) and o-nitrobenzaldehyde (LXXIV). Another reason for attempting this reaction was that the product might well be converted into an "aristolochic acid" in which the positions of the nitro and carboxylic acid groups were reversed (LXXVI).

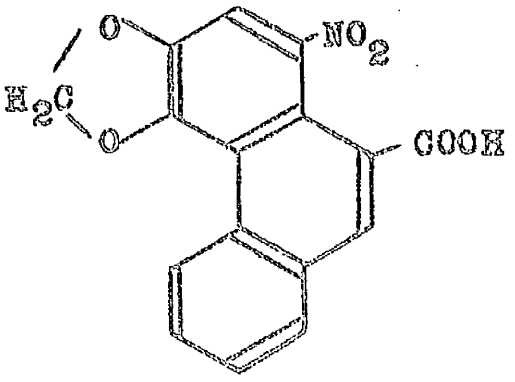


(LXXIII) (LXXIV)

(LXXV)

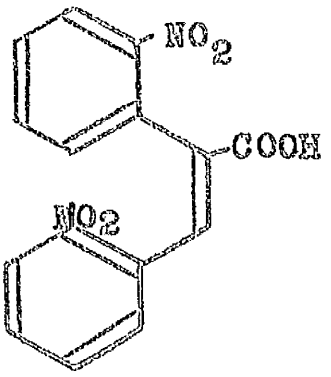
6-Nitrohomopiperonylic acid (LXXIII) was prepared in a good yield according to the method of Tiermann[109], but the method of Percy and Robinson[110] failed in our hands. Various conditions for condensation were tried including acetic anhydride and ethanol as solvents and the acid in the form of acid, ester or sodium salt both with and without a catalyst. Only with the sodium salt in acetic anhydride was a crystalline product obtained but this

proved to be o-nitrocinnamic acid and was confirmed by comparison with an authentic sample in mixed melting point and infrared absorption spectrum.



(LXXVI)

This compound must arise because of traces of acetate or acetic acid in the anhydride. At room temperature for three weeks both in acetic anhydride and in quinoline starting material was isolated. A summary of the results obtained is given in Table 6. The reaction of the ethyl ester of 6-nitrohomopiperonylic acid and nitrobenzaldehyde in ethanol with sodium as catalyst was based on the method of Reissert[111] for the synthesis of compound (LXXVII). Reissert isolated this compound in good yield, but considerable decomposition occurred in our hands under similar conditions and dark red oily material was isolated which did not crystallise.



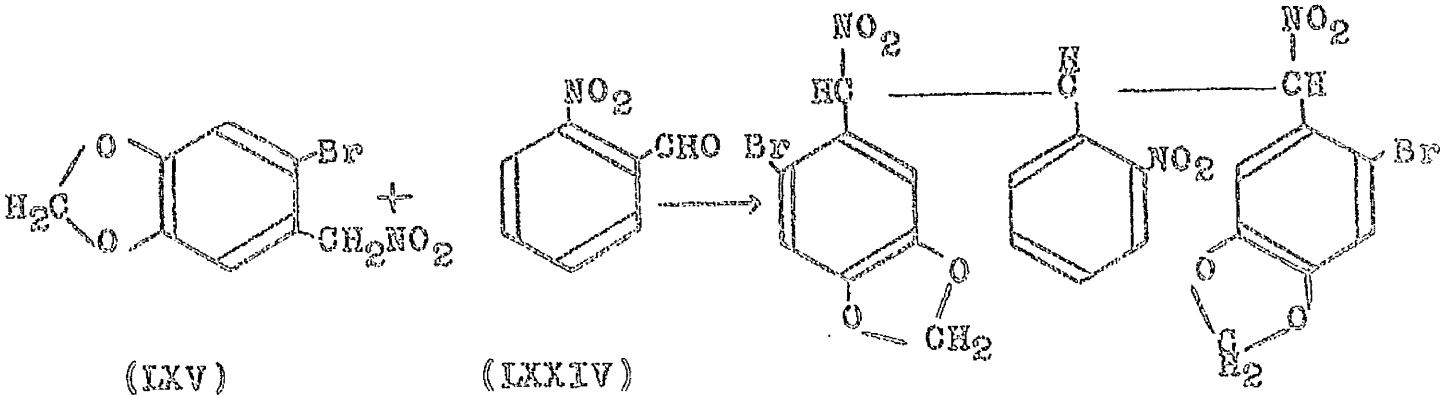
(LXXVII)

An interesting reaction occurred when a few drops of triethylamine were added to a solution of the acid and o-nitrobenzaldehyde in acetic anhydride. Immediately a bulky shining red crystalline mass separated which was filtered off, washed with a little acetic anhydride and dried at room temperature, but the crystals gradually decomposed. Later it was found that triethylamine itself reacted with 6-nitrohomopiperonylic acid to yield an unstable compound which decomposed even in solution. Although these results were not encouraging, 6-bromo-3,4-methylenedioxyphenylnitromethane was heated with o-nitrobenzaldehyde in acetic anhydride for 10, 15 and 20 hours. Starting material was recovered from these experiments and it appeared likely that a basic catalyst, or a basic solvent, would be required, as in the condensation of phenylnitromethane and benzaldehyde[76].

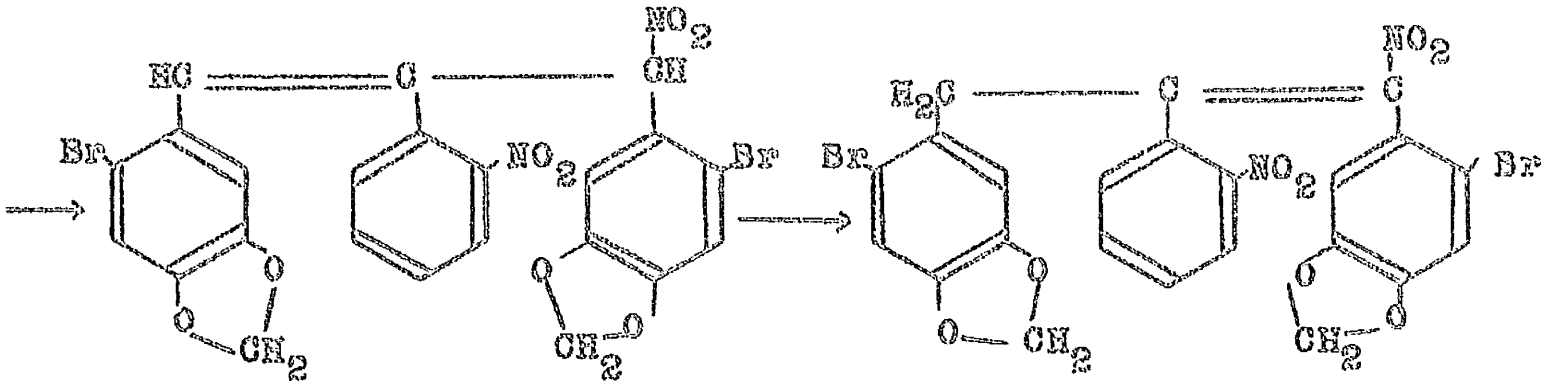
Hass, and Riley[112] in their review reported that a basic catalyst increases the concentration of aci-nitroparaffin by exerting its well known catalytic effect in restoring the tautomeric equilibrium after the trace of nitronic acid normally present has reacted with aldehyde.

With acetic anhydride and triethylamine as a catalyst white crystals, m.p. 200 -202°, were obtained and identified as crude o-nitrocinnamic acid. Fraser and Kon[113], and Nightingale, Erickson and Knight[114] used ethanol as solvent in the presence of sodium ethoxide but their conditions yielded 6-bromopiperonaldehyde as the only identifiable product, and considerable decomposition also occurred. Ethylamine, as used by Knoevengel and Walter[76] gave more interesting results and white crystals were obtained which on recrystallisation from hot ethanol yielded the starting material 6-bromo-3,4-methylenedioxyphenylnitromethane. Analysis of the crystals as isolated from the reaction mixture indicated a formula of $C_{10}H_{13}O_4N_2Br$ which on subtraction of $C_8H_6O_4N$ Br (starting material) left a residue of C_2H_7N or ethylamine. Aliphatic nitro-compounds are pseudo acids and the crystals here are in all probability an unstable compound of the base and nitro-compound. Under the conditions of recrystallisation it might well decompose to give starting material as found.

Attempted condensation was carried out under the following conditions:- In pyridine at room temperature for 10 days, ethanol containing triethylamine or n-butylamine but all yielded a white crystalline material m.p. 198-200°. The melting point of the crystals (Compound A, Table 7, page 161) was similar to that for o-nitrocinnamic acid, but the infrared absorption curve of the compound was completely different, particularly in respect of the enhanced absorption of the aromatic band of 1620cm^{-1} which could well be due to the presence of an ethylenic bond. Analysis of the compound indicated a composition of $C_{23}H_{14}O_8N_2Br_2$ although this must be accepted with some reserve as the percentage of carbon found was 46.2 as compared with a required 45.4%. A compound (LXXVIII) or (LXXIX) of this formula can however be obtained easily by the following logical sequence of reactions, based upon Heim's work[115] on the preparation of α'-nitrostilbenes.

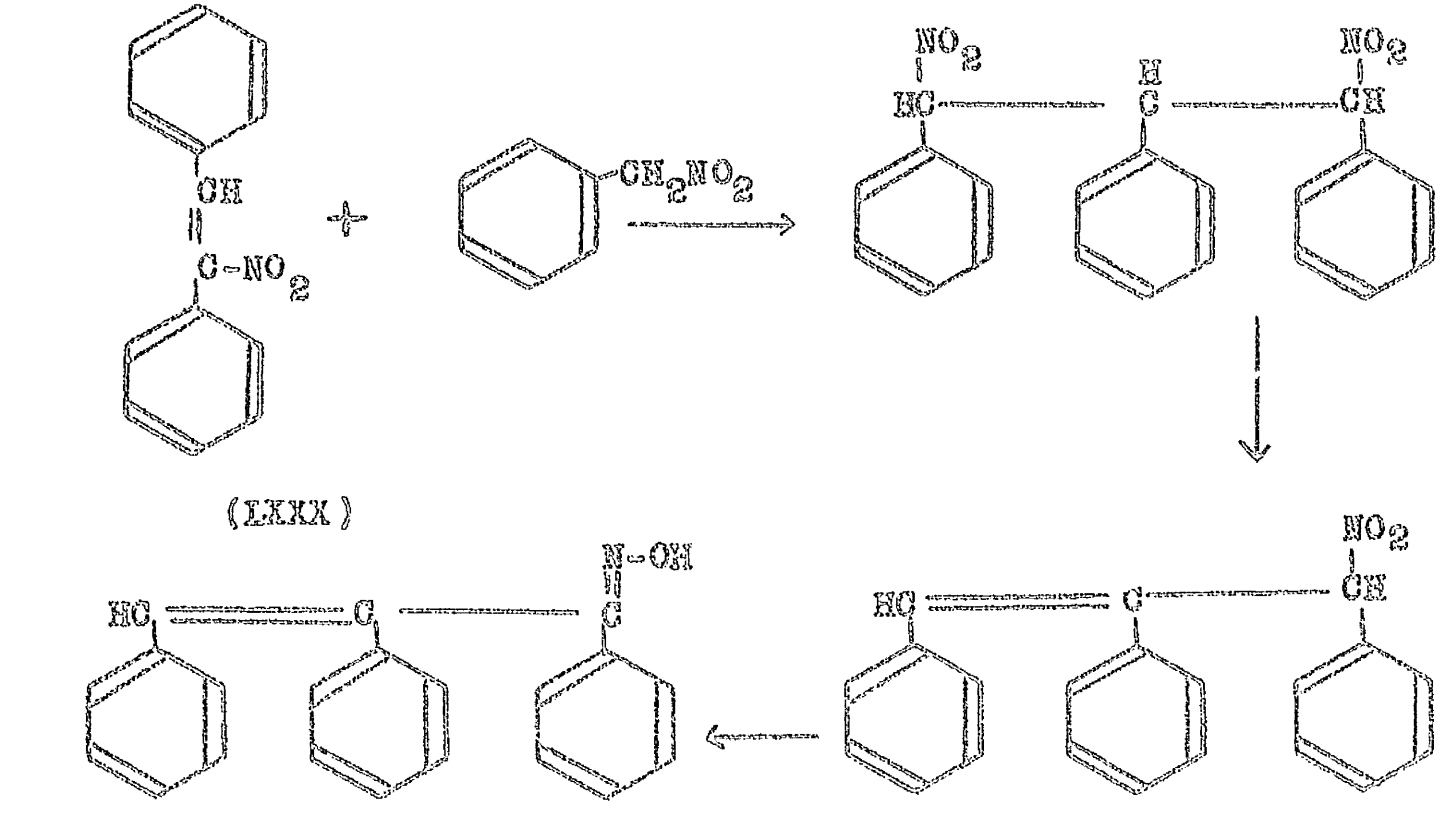


(LXV) (LXXIV)



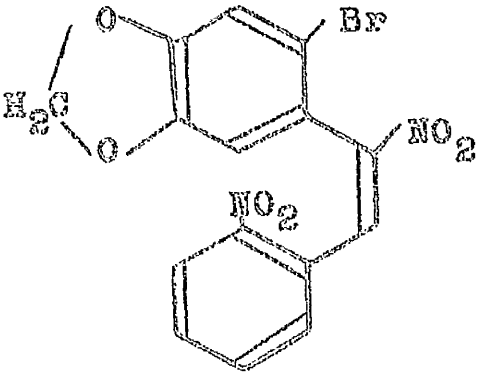
(LXXVIII) (LXXIX)

This is in agreement with the reaction of α-nitrostilbene (LXXX) in the presence of phenylnitromethane under the influence of ethanol and ammonia as observed by Worrall[116].



(LXXX)

The reaction could therefore proceed by direct condensation of 2 moles of the nitrocompound (LXV) with one of nitrobenzaldehyde (Helm)[115] or by the addition of unreacted nitrocompound to the required condensation product (LXXXI).



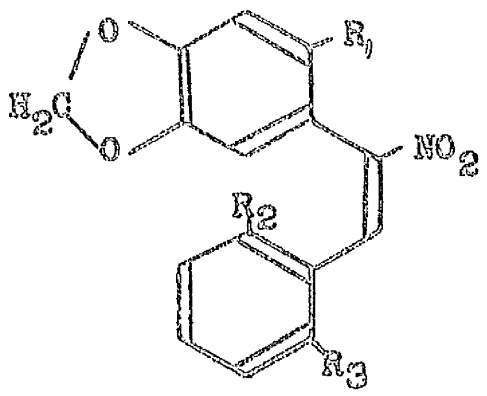
(LXXXI)

By this second mechanism the analogy with Worrall's observation is close. With the solvent systems quinoline, pyridine and pyridine plus diethylamine, 6-bromopiperonaldehyde was isolated along with some unidentified nitrile or isocyanide (infrared absorption spectra showed the characteristic nitrile absorption at 2226cm^{-1}). When piperidine was used only 6-bromopiperonaldehyde was isolated and no nitrile or isocyanide was formed. The course of the reaction involving nitrile formation is clearly associated with the aromatic solvents. Lack of information concerning the identity of the nitrile prevents the presentation of a

tentative mechanism.

Emerson[117] in his review reported the use of various condensing agents for a variety of compounds under various conditions, but no evaluation of the various methods to ascertain the most generally useful procedure seems to have been made[118,119]. Some considerable time was therefore spent in investigating suitable conditions for the required condensation and a summary of the unsuccessful experiments is given in table 7 (page 161).

Satisfactory condensation to the required α-nitro-6-bromo-3,4-methylenedioxy-2′-nitrostilbene (LXXXII, R_1=Br, R_3=H, R_2=NO_2) was eventually achieved in ethanol as solvent with a trace of ethylamine as a catalyst at room temperature for two weeks.



(LXXXII)

In the same way the following compounds were prepared:-

α-Nitro-3,4-methylenedioxy-2′-nitrostilbene (LXXXII, R_1=R_3=H, R_2=NO_2),

α-Nitro-6-bromo-3,4-methylenedioxy-6′-methoxystilbene (LXXXII, R_1=Br, R_2=H, R_3=OCH_3),

α'-Nitro-6-bromo-3,4-methylenedioxy-2'-nitro-6'-methoxystilbene (LXXXII, R_1=Br, R_2=NO_2, R_3=OCH_3),

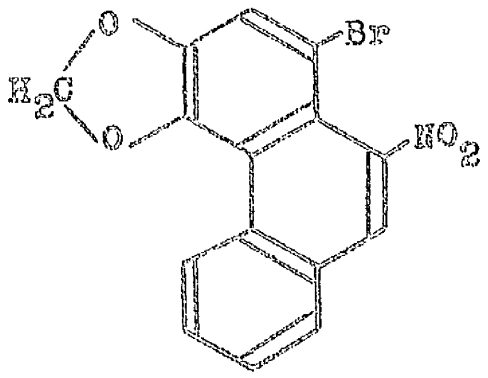
α-Nitro-3,4-methylenedioxy-2'-nitro-6'-methoxystilbene (LXXXII, R_1=H, R_2=NO_2, R_3=OCH_3),

thus utilising the intermediate (XXXVIII) which had already been prepared earlier.

REDUCTION OF NITROCOMPOUNDS

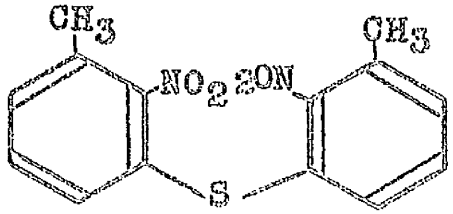
Among the many reducing agents used in organic chemistry ammonium sulphide is one which often selectively reduces one of two nitro groups. Generally but not always[120] the groups are attached to one ring and in the present instance ammonium sulphide was selected as first choice.

Reduction of α-nitro, 6-bromo-3,4-methylenedioxy-2′ nitro-stilbene, (LXXXII, R_1=Br, R_2=NO_2, R_3=H) proceeded smoothly in ammoniacal solution with sufficient ammonium sulphide to reduce one nitro group, and a 33% yield of bright yellow crystals was obtained. The infrared absorption curve indicated the presence of a nitro group (1510cm^{-1}) and the compound analysed for $C_{15}H_{10}NO_4Br$, i.e, it appeared as if one nitro group had been removed. Although the ultraviolet absorption curve (fig. A) showed a similarity to that of 9-nitrophenanthrene (see below), it appeared unlikely that the compound was in fact the phenanthrene derivative (LXXXIII).



(LXXXIII)

Ammonium sulphide is known to eliminate a nitro group with the formation of sulphide[121,122] (LXXXIIIa) but sulphur was absent from our compound.



(LXXXIIIa)

It therefore remained to decide which nitro group had been eliminated. Comparison of the ultraviolet absorption curves of the parent (fig. B) and reduced compound (fig. A) indicated a marked change in the chromophoric system but unfortunately did not provide conclusive evidence of the structure of the reduced compounds. The parent compound is made up of several chromophoric systems as indicated by the partial structures (LXXXIV) , (LXXXV), (LXXXVI)and (LXXXVII) and a great deal of crossed conjugation exists. Consequently the absorption curve is likely to be complex. Of these systems that of nitrostyrene (LXXXV) is probably of major importance in agreement with Freeman and Stevens[123] work on the α-nitrostilbenes. The situation is complicated by geometrical isomerism in the styrene and stilbene systems, and the additional methylenedioxy groups on the stilbene system.

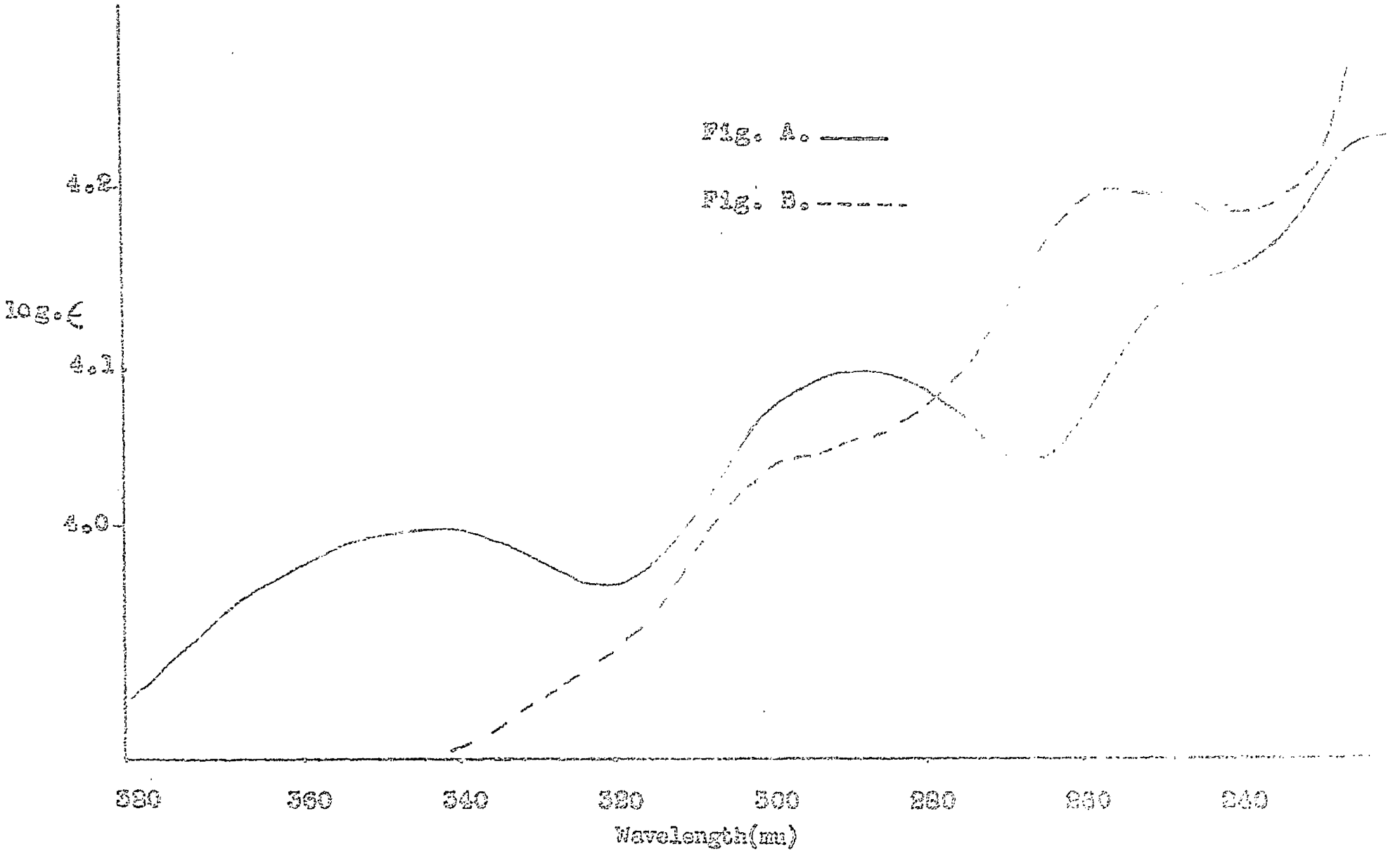
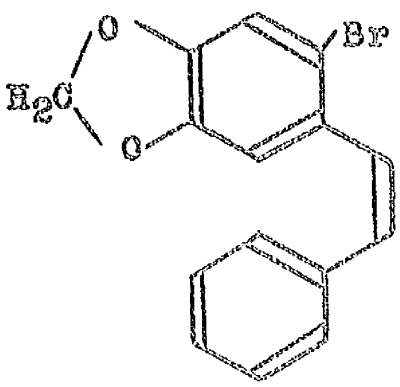
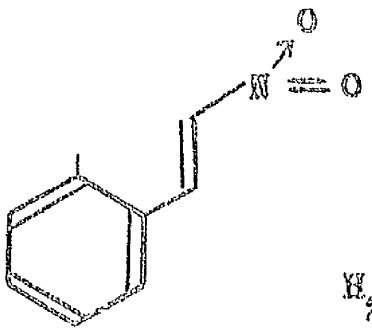


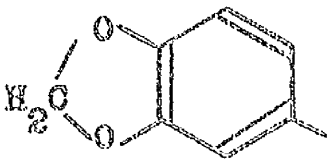
Fig. A. ——
Fig. B. - - - -
4.2
log.ε
4.1
4.0
380
360
340
320
300
280
260
240
Wavelength(mμ)



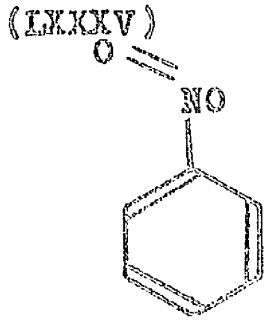
(LXXXIV)



(LXXXV)



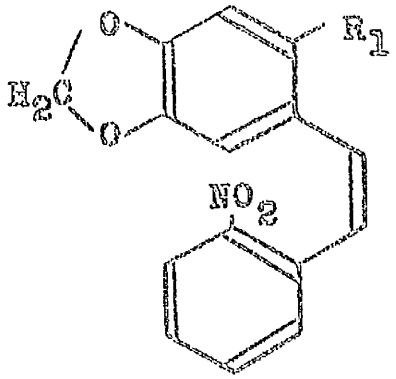
(LXXXVI)



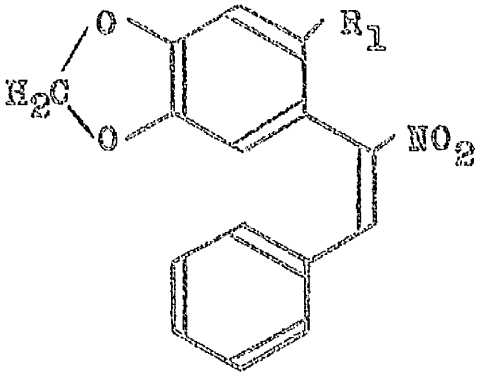
(LXXXVII)

By analogy with the normal condensation of phenylacetic acids and nitrobenzaldehydes leading to the cis stilbene system under the influence of the carboxyl group[124], the stilbene system in our compounds should be cis also because of the nitro group. This is not conclusive because Helm[115] isolated both cis and trans α-nitrostilbene when condensing phenylnitromethane with benzaldehyde. In any event the observed ultraviolet absorption curve (fig. A) is not unexpected in showing lack of absorption above 300mμ. Reduction, however, introduces a marked change in the absorption curve indicative of the removal of crossed conjugation.

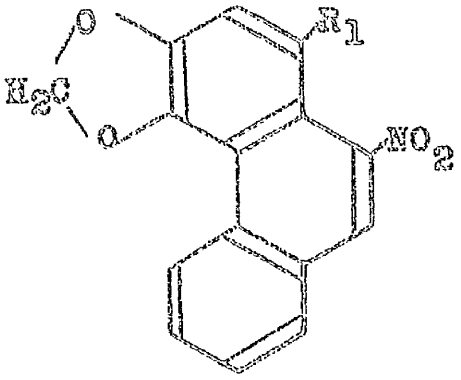
The loss of one nitro group may occur to give several possible compounds (LXXXVIII)(LXXXIX)(XC)and(XCI)



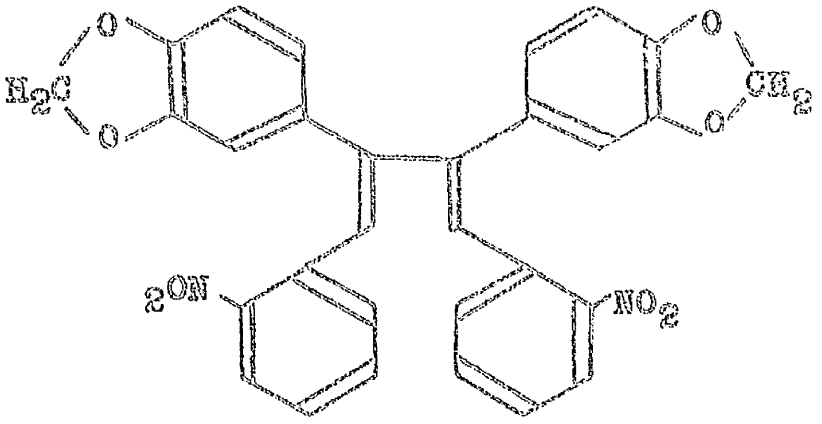
(LXXXVIII)



(LXXXIX)



(XC)



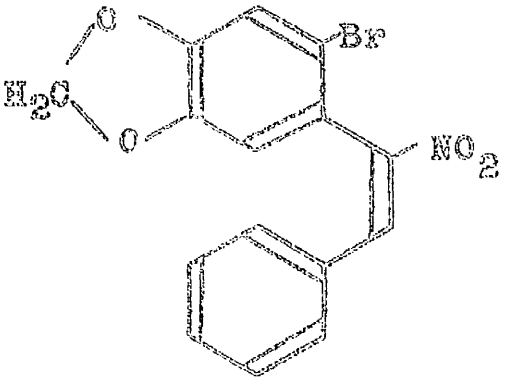
(XCI)

Loss of the aromatic nitro-group leading to compound (LXXXIX) would not account for a marked change because Reagan and Brown[125] has shown that ortho-substitution tends to hinder the resonance of the nitro group and consequently its effect on the absorption curve is small. A bathochromic shift might be expected with compound (LXXXIX) although it must be admitted that the work of Detar and Carpino[126] on the 2-nitrostilbenes is somewhat

in conflict with this view in that the cis-phenyl compound has λ max 260 mu (log ϵ 4.11) and the trans- phenyl compound has λ max 274 mu (log ϵ 4.30). Our own compound should correspond to the cis-phenyl stilbene system but the authors do not indicate that there was any absorption above 315-320 mu. The presence of the methylenedioxy group would induce a bathochromic shift of the absorption but it is unlikely to cause such a marked change unless the stilbene system is trans (c.f. 4-methoxy-cis-stilbene λ max 285, log 4.2) and 4-methoxy-trans stilbene λ max 305 and 320, 335 mu, log ϵ 4.5, 4.5 and 4.3 respectively[127]. The most satisfactory compound that would account for the observed absorption is (XC) [c.f. 9-nitrophenanthrene, λ max 248.5, 290 and 332 mu, log ϵ 4.66, 3.98 and 3.82 respectively[128]] but this is disproved in that compound (XC, R=H) is known, being prepared by decarboxylation of aristolochic acid II. Compound (XCI) although possessing crossed conjugation is also likely to absorb well above 300 mu. No evidence is, however, available to support either the reaction leading to compound (XC) or to compound (XCI). On the other hand removal of the α-nitro group would eliminate the crossed conjugation and it appeared therefore desirable to confirm this tentative conclusion by chemical means.

Condensation of benzaldehyde with 6-bromo-3,4-

methylenedioxyphenylnitroethane afforded a compound presumably (XCII) m.p. 119-120° which was quite different from that isolated from the reduction of compound (LXXXII, R_1=Br, R_2=NO_2, R_3=H) with ammonium sulphide.

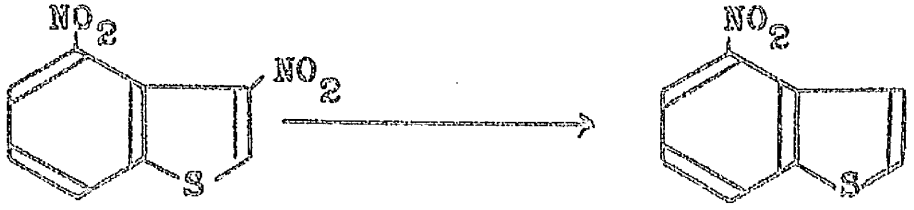


(XCII)

The product isolated must therefore be as shown (LXXXVIII, R_1=Br). More satisfactory confirmation was obtained when 6-bromohomopiperonylic acid was condensed with o-nitrobenzaldehyde and the product decarboxylated with quinoline and copper powder. Bright yellow crystals were isolated, although in low yield and comparison of the infrared absorption curve of the crystals with that of the product obtained by reduction showed complete identity. Thin layer chromatography using petroleum ether and diethyl ether (1:1) as solvent and sulphuric acid 50% as detecting agent was also used to confirm their identity. The product isolated from the reduction of compound (LXXXII, R_1=Br, R_2=NO_2, R_3=H) with ammonium

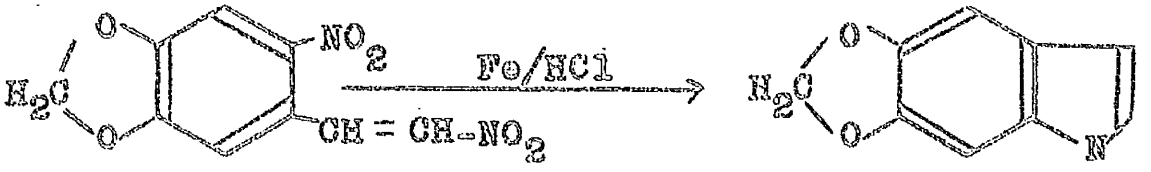
sulphide was further reduced with zinc and dilute hydrochloric acid. The product was diazotised and yielded a bright red colour with alkaline β-naphthol.

α-Nitro, 3,4-methylenedioxy-2′-nitrostilbene yielded with ammonium sulphide a product similar to that obtained above and although the analysis for carbon is not entirely satisfactory, there can be little doubt that it is represented by (LXXXVIII, R_1=H). The complete loss of the nitro-group with replacement by hydrogen is unusual as the mode of reaction is generally the formation of a sulphide or disulphide as investigated by Hodgson and Ward[129]. Unfortunately aromatic nitro compounds only have been reduced with sulphide and no reference to this technique as being applied to aliphatic nitro-compounds has so far been found. The nearest reaction to that observed in the present work is that of Fries, Heering, Hemmecke and Siebert[130] who reduced the dinitrothionaphthen (XCIII) with ammonium sulphide and obtained the 4-nitro compound (XCIV). Elimmation of a nitro group from a nitro-olefin was also observed by Burton and Duffield[131] in the course of their synthesis of 5,6 dihydroxyindole.



(XCIII) (XCIV)

This example is however not so satisfactory since it is not clear which nitro group is eliminated.



Loss of a nitro-group also occurs with α-nitrostilbene in the presence of ethanol and ammonia a reaction which has already been discussed on page 120. The analytical figures for the product, however, preclude this type of reaction from having taken place.

In view of the results discussed above other reducing agents tried but without any success were; catalytic hydrogentation[132,133] of α-nitro -6-bromo-3,4-methylenedioxy -2′-nitrostilbene to yield a mixture of four components as shown by thin layer chromatography; iron powder and hydrochloric acid, which has been reported[134] to be a better method for the reduction of aromatic nitro groups, yielded starting material and a compound containing a nitrile group; and the other reagents summarised in Table 8, (page 169).

Attempted reduction with ammonium sulphide of the compounds containing the methoxyl group (LXXXII, R_1=Br, R_3=OCH_3, R_2=NO_2) and (LXXXII, R_1=H, R_2=NO_2, R_3=OCH_3)

yielded starting material only. Using double the theoretical amount of ammonium sulphide some pale yellow crystals were obtained but unfortunately in too small a quantity for purification. The infrared absorption curve of the impure product indicated that nitro groups were absent and Lassaigne's test also showed the absence of nitrogen. The presence of the methoxyl group in these compounds appeared to affect the course of the reduction with ammonium sulphide and no satisfactory material was isolated.

The lack of success in the reduction experiments prompted an examination of condensation with acetyl-aminobenzaldehyde. 3,4-Methylenedioxyphenylnitromethane and the 6-bromo-compound were treated with o-acetyl-aminobenzaldehyde under the conditions for which the condensation with the nitrobenzaldehyde proceeded smoothly. No similar reaction occurred even over a period of 14 days, and the method of Worrall[135] for the preparation of nitrostyrene also yielded starting material. This condensation clearly requires a period of intensive investigation.

In order to complete the examination of the route which it was hoped would lead to aristolochic acid, attempts were made to introduce the carboxyl group into 6-bromo-3,4-methylenedioxy-2′-nitrostilbene (LXXXVIII, R_1=Br). Heating with a mixture of silver and cuprous cyanide in the presence of copper sulphate and pyridine failed to produce any reaction. At the higher reflux temperature of quinoline, however, a small yield of a substance m.p. 191-192° was isolated. The analytical figure for carbon differs appreciably from the expected value (65.84% found; 65.3% required) but the infrared absorption indicated the presence of the nitrile group. This result was encouraging in view of the complete failure of the experiments discussed on page 99 .

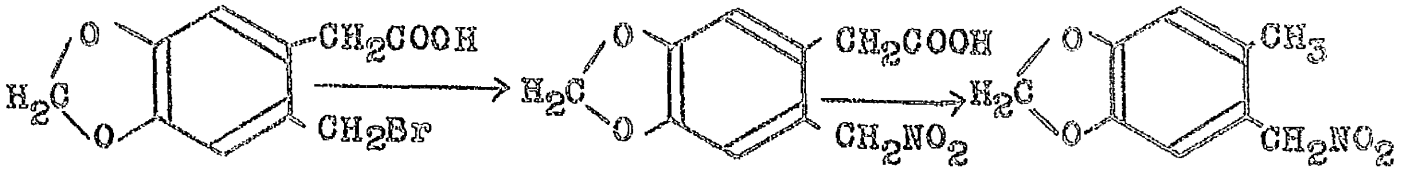
The Grignard reaction using compound (LXXXII, R_1=Br) was attempted but no reaction took place and a similar result was obtained when lithium was used in place of magnesium. The entrainment method[136] using magnesium methyl iodide as an activator gave a highly fluorescent solution. Evaporation of the solvent yielded a mixture of pale yellowish brown crystals (non-fluorescent) which could not be recrystallised for further investigation. Infrared absorption showed the absence of nitro groups but both nitrogen and bromide were still present (Lassaigne's and Beilstein's test). It is well known that Grignard

reagents react with nitro-compounds to yield a mixture of compounds[137,138] depending upon the nature of the starting material and this method may well be inapplicable as a means of introducing the carboxyl group.

Mallory, Wood and Gordon[139] have recently shown that the synthesis of substituted phenanthrene compounds may be achieved by irradiation of stilbenes in cyclohexane using iodine as a catalyst. The authors point out that the reaction fails with nitrostilbenes but do not indicate the actual compounds examined. It therefore appeared reasonable to prepare and irradiate compounds (LXXXII, R_1=H, R_2=H, R_3=OCH_3) and (LXXXVIII, R_1=Br) on the micro-scale and to examine the ultraviolet absorption curves of the resultant solutions for evidence of formation of phenanthrene compounds. In both tests a rapid change occurred in the absorption spectra but no absorption typical of phenanthrene was obtained.

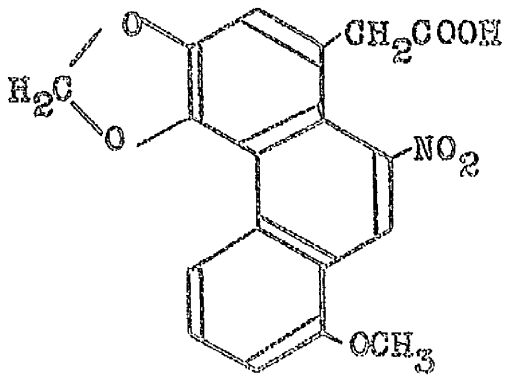
Conclusions

The postulated route to aristolochic acid has been shown to be fraught with difficulties yet it may still be feasible providing two important steps can be accomplished. These are the condensation of acetylaminobenzaldehyde and its derivatives with the nitro-compounds and the preparation of the intermediate (XXXIX). The latter step may not, in fact, be essential because a methyl group suitable for oxidation to the carboxyl group at the last stage of the synthesis can be introduced as follows:-



(XCV) (XCVI) (XCVII)

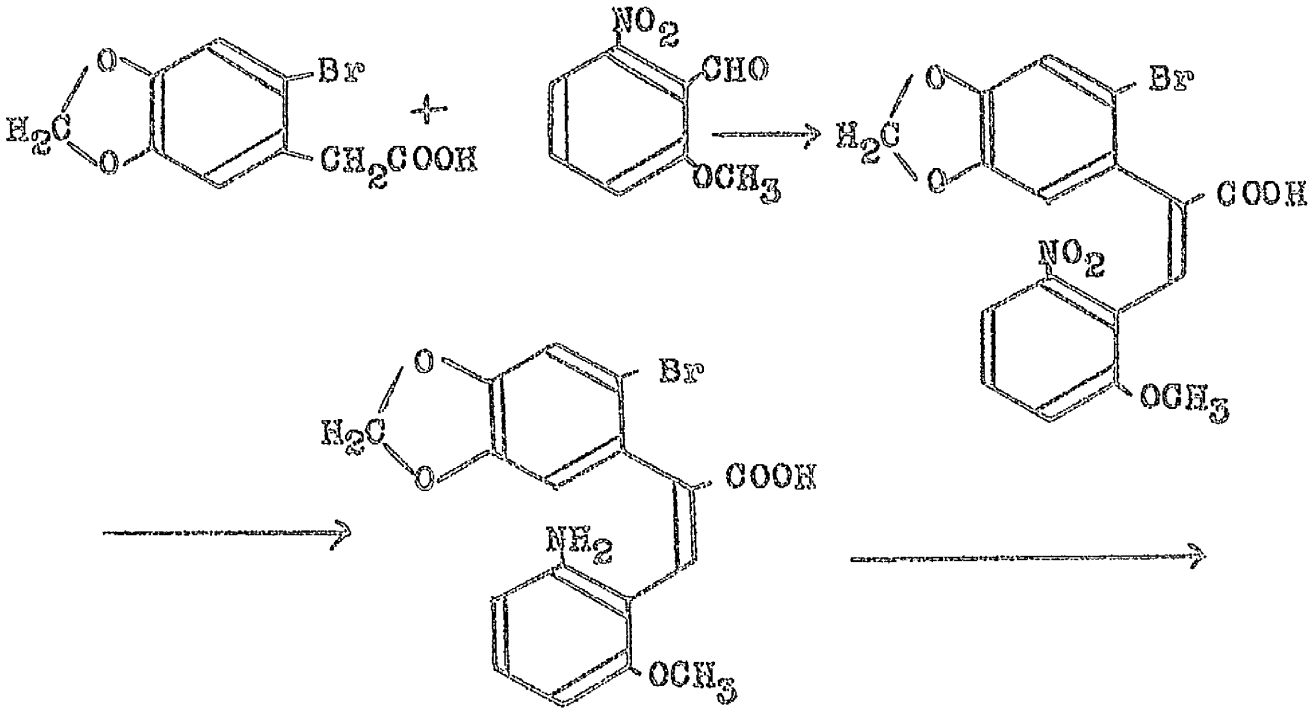
Compound (XCV) was prepared as described by Stevens[80] for this purpose but time did not permit an extension of the work. Compound (XCVI) is perhaps doubly important in that it could well be used for the synthesis of debilic acid (XXX) although several products are likely to arise in the condensation with the substituted benzaldehydes. Also, as described earlier, although the parent hydroxyacid could not be isolated, the lactone did give compound (XCV) with hydrogen bromide and it is difficult to see why intermediate (XLIX, page 98) could not be obtained in the same way.

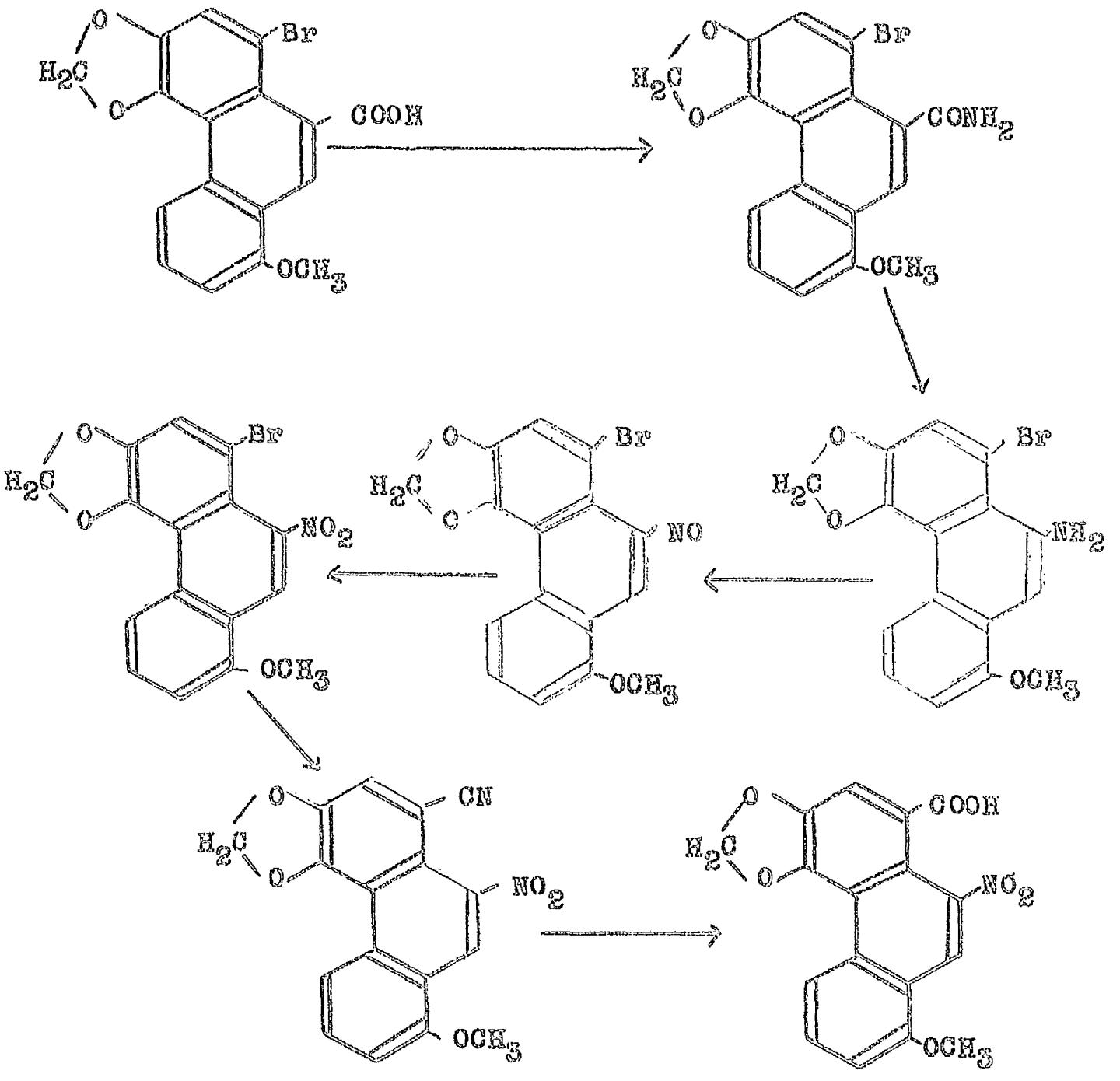


(XXX)

The experiments so far carried out involved the introduction of the nitro-group at an early stage of the synthesis and it has been shown that considerable difficulty arose because of this. Consideration must therefore be given to methods in which the nitro-group is introduced as a final step.

This may conceivably occur as follows:-





The failure of this route at any stage must necessarily lead to the use of naphthalenic intermediates and an entirely new approach to the synthesis of aristolochic acids.

EXPERIMENTAL

M.Ps. are uncorrected. Ultraviolet absorption spectra were determined in absolute ethanol on Optica D.F.4 Recording Spectrophotometer. The author is indebted to Dr. A.C. Syme and Dr. Proctor of the Chemistry Department of this University and Dr. G. Weller and Dr. F.B. Strauss (Oxford) for the microanalyses.

ISOLATION OF ARISTOLOCHIC ACID

The dried root and rhizome of A. indica were reduced to a No.60 powder (6.5Kg) defatted with light petroleum (40-60) (4 days) and percolated in the cold with ethanol until the percolate was pale brown (7days). Concentration of the percolate to 200 ml under reduced pressure gave an almost black, thick oil. The marc was further extracted in a large Soxhlet extractor with hot ethanol; the hot percolate on concentration to 100 ml gave a black, thick oil which was mixed with the oil from the cold percolation. The oil, on standing overnight in a refrigerator deposited β-sitosteryl-β-D-glucoside (1.2g) as a brown crystalline solid m.p. 285-292°, which on recrystallisation from ethanol (charcoal) gave white microcrystals m.p. 294-6°. The mother liquor obtained after separation of the glucoside was acidified with dilute hydrochloric acid and extracted with ether repeatedly until the ether layer was almost colourless. The ethereal solution was extracted with 4% aqueous sodium bicarbonate and the latter on acidification yielded crude aristolochic acid (5.6g) which was recrystallised from dioxan as a bright yellow micro crystalline solid, m.p. 284° (decomp.).

DERIVATIVES OF ARISTOLOCHIC ACID

Lactam

Aristolochic acid (1g) was refluxed for 45 minutes with zinc powder (5g) and glacial acetic acid (60ml). The fluorescent solution was filtered whilst hot, and on cooling, the bulk of the crude product (600mg) was deposited. The mother liquor on treatment with water (1500ml) yielded a further precipitate, which was extracted with chloroform. The bulked chloroform extracts were washed repeatedly with water, dried (Na_2SO_4) and evaporated to give a further 100mg of the crude product. Recrystallisation of the solid gave highly fluorescent greenish-yellow crystals of 10-amino-8-methoxy-3,4-methylenedioxy-1-phenanthroic lactam, m.p. 319-320° (microblock). Coutts[20] gave m.p. 320° (inserted at 315°). Pailer[2] gave m.p. 319°.

Lactam Acetate

10-Amino-8-methoxy-3,4-methylenedioxy-1-phenanthroic lactam (300mg) was refluxed for 30 minutes with acetic anhydride (2ml) and pyridine (3ml). The yellow precipitate which separated during the reaction and on cooling yielded 10-acetamido-8-methoxy-3,4-methylenedioxy-1-phenanthroic lactam (200mg) as greenish yellow needles,

which fluoresced under ultraviolet light both in the solid state and in solution, m.p. 293-295° (decomp.) Coutts[20] gave m.p. 295° (decomp.), Rosenmund and Reichstein[4] gave m.p. 292-296° (decomp.), for the so-called diacetate.

Lactam Benzoate.

10-Amino-8-methoxy-3,4-methylenedioxy-1-phenanthroic lactam (150mg) was refluxed for two hours with benzoyl chloride (120mg) and dry pyridine (5ml). On cooling in a refrigerator overnight, most of the benzoate separated. The mother liquor yielded a further 20 mg of product on concentration *in vacuo* on a boiling water bath. Recrystallisation of the solid from chloroform gave greenish-yellow needles of the lactam benzoate m.p. 325-326° (decomp.), which fluoresced under ultraviolet light both in the solid state and in solution.

Found:	C, 72.88;	H, 4.27;	N, 3.8%
$C_{24}H_{15}NO_5$ requires:	C, 72.54;	H, 3.77;	N, 3.8%

Attempted Preparation of Lactam Succinate

The lactam was heated with the reagents under various conditions, which are recorded, with results, in Table 3.

T A B L E 3.

Lactam (mg)	Reagents	Time (Hrs)	Bath Temperature. (C)	Results.
300	Succinic anhydride (120mg) Dry Pyridine (6ml)	2	130-140	Lactam isolated.
300	" "	5	150	Lactam and succinic anhydride isolated.
300	" "	6	180	Lactam and succinic anhydride isolated.
150	Succinic anhydride (50mg)	2	150	Succinic anhydride sublimed.
150	Methyl succinyl chloride (200mg) pyridine (4ml)	3	140	Lactam isolated.
150	" "	5	180	"
150	" and tetrahydrofuran	4	170	"

Attempted Preparation of Lactam Phthalate

The experiments summarised in Table 3 were also carried out using phthalic anhydride and methyl phthalyl chloride[141] in place of the corresponding succinic compounds. Lactam was recovered in every experiment.

2-METHOXY-6-NITROBENZALDEHYDE.

2-Hydroxy-6-nitrobenzaldehyde was prepared from m-nitrophenol by the method of Shirai and Oda[73] in 3% yield, m.p. 53-54°. Ashley, Perkin Jr., and Robinson[72] found m.p. 54-55° yield 3%. Shirai and Oda found m.p. 53-54°.

2-Methoxy-6-nitrobenzaldehyde was prepared by the methylation of 2-hydroxy-6-nitrobenzaldehyde with dimethyl sulphate by the method of Shirai and Oda[73] in 65% yield m.p. 110-111°, literature m.p. 111°.

3,4-METHYLENEDIOXY-6-HYDROXYMETHYLBENZOIC ACID

6-Bromopiperonaldehyde was prepared from piperonaldehyde by the method of Orr, Robinson and Williams[77] in 80% yield, m.p. 128-129°, literature m.p. 129-130°.

6-Bromohomopiperonyl alcohol was prepared by the reduction of 6-bromopiperonaldehyde with lithium aluminium hydride by the method of Nalk and Wheeler[78] in 95% yield, m.p. 88-89°, literature m.p. 89-90°.

Attempted preparation of 6-cyanohomopiperonyl alcohol.

(I) 6-Bromohomopiperonyl alcohol (2g) was refluxed for 10 hours with dry copper cyanide (1g) and dry pyridine (15ml). The mixture was diluted with water (50ml)

and extracted with ether (100ml x 3). The ethereal layer was extracted with ice-cold dilute hydrochloric acid, washed with water and dried (Na_2SO_4). Evaporation of the ether gave 6-bromohomopiperonyl alcohol m.p. 87-89°, undepressed on admixture with starting material.

(II) The experiment was repeated using reflux times of (a) 15 hours and (b) 24 hours with the addition of dry potassium cyanide and a small quantity of anhydrous copper sulphate; 6-bromohomopiperonyl alcohol was recovered.

(III) 6-Bromohomopiperonyl alcohol (3g) was heated on an oil bath (135-145°) for 10 hours with quinoline (15ml) and copper cyanide (1.5g). The reaction mixture was treated by the method of Helberger and Rebay[142], but 6-bromohomopiperonyl alcohol was recovered.

(IV) The experiment was repeated using temperatures of (a) 160-170° and (b) 200-205° for 10 hours with the addition of dry powdered potassium cyanide (0.5g) and anhydrous copper sulphate. Decomposition occurred in both experiments.

Piperonylcyanohydrin was prepared from piperonaldehyde as a pink oil by the method of Barger and Ewins[79] in 80% yield.

3,4-Methylenedioxyphenylhydroxyacetiminoethylene-ether hydrochloride was prepared from piperonylcyanohydrin by the method of Barger and Ewins[79] in 55% yield, m.p. 119-120°, literature m.p. 118-119°.

Ethyl-3,4-methylenedioxymandelate was prepared from 3,4-methylenedioxyphenylhydroxyacetiminoethylene-ether hydrochloride by the method of Barger and Ewins[79], m.p. 70-72°, literature m.p. 72°.

Homopiperonylic acid was prepared from ethyl-3,4-methylenedioxymandelate by the method of Stevens[80] in 70% yield, m.p. 128°, literature m.p. 127°.

6-Hydroxymethylhomopiperonylic acid lactone was prepared from homopiperonylic acid by the method of Barger and Ewins[79] as colourless leaflets m.p. 136°, literature m.p. 137°.

6-Bromomethylhomopiperonylic acid was prepared from the lactone of 6-hydroxymethylhomopiperonylic acid by the method of Stevens[80], m.p. 145-147°, literature m.p. 146-149°.

α-(6-Hydroxymethylpiperonyl) cinnamolactone was prepared from 6-hydroxymethylhomopiperonylic acid lactone by the method of Stevens and Robertson[81] as pale yellow needles in 70% yield, m.p. 191°, literature m.p. 190-192°.

4,5-Methylenedioxyphthalide was prepared from α-(6-hydroxymethylpiperonyl) cinnamolactone by the method of Stevens and Robertson[81], m.p. 190°, literature m.p. 188-189°. It readily sublimed at 150-160° without decomposition.

Attempted preparation of 6-bromomethylpiperonylic acid

4,5-Methylenedioxyphthalide (1g) was dissolved in glacial acetic acid (15ml) and treated with dry hydrogen bromide according to the method of Stevens[80] for the preparation of 6-bromomethylhomopiperonylic acid but starting material was isolated.

3,4-Methylenedioxy-6-hydroxymethylbenzoic acid was prepared from 4,5-methylenedioxyphthalide by the method of Stevens and Robertson[81] as white micro-crystals which melted indefinitely at 145-150°, quickly resolidified and then melted at the m.p. of the lactone 188-189°.

ATTEMPTED PREPARATIONS WITH 3,4-METHYLENEDIOXY-6-HYDROXYMETHYLBENZOIC ACID.

6-Bromomethylpiperonylic acid.

(I) 6-Hydroxymethylpiperonylic acid (1g) was dissolved in dry ethanol (25ml), cooled in a freezing mixture and hydrogen bromide[143] gas was bubbled through at a temperature below 0°. Immediately white shining crystals separated and these were filtered, washed with a little ethanol and identified as 4,5-methylenedioxyphthalide by mixed m.p. 188-189° and infrared absorption.

(II) 6-Hydroxymethylpiperonylic acid (1g) was refluxed in dry ethanol (20ml) whilst hydrogen bromide was bubbled through the solution for one hour. On cooling, white shining needles of 4,5-methylenedioxyphthalide separated.

(III) Finely powdered 6-hydroxymethylpiperonylic acid (1g) was shaken vigorously with aqueous hydrobromic acid (50% W/V) (10g) as described by Robinson and Robinson[144]. The crystals, which separated, were washed with a little ethanol and identified as 4,5-methylenedioxyphthalide.

6-Hydroxymethylethylpiperonylate.

(I) 6-Hydroxymethylpiperonylic acid (400mg) was dissolved

in absolute ethanol (20ml), neutralised with 10% sodium hydroxide solution using phenolphthalein as indicator. The sodium salt of the acid was treated with a small excess of 10% silver nitrate solution till greyish white micro-crystals precipitated. The precipitate of silver salt was filtered, washed with a mixture of water and ethanol (1:1) and the salt was refluxed for one hour with ethanol (10ml) and ethyl iodide (0.5ml). The precipitate which formed was filtered off and the filtrate on cooling deposited 4,5-methylenedioxyphthalide.

(II) The silver salt of the acid was shaken with methyl iodide at room temperature for 24 hours, but 4,5-methylenedioxyphthalide separated after removing the silver salt.

(III) 6-Hydroxymethylpiperonylic acid (200mg) was refluxed for 2 hours with absolute ethanol (5ml) and one drop of sulphuric acid. As soon as the acid was added white shining needles of 4,5-methylenedioxyphthalide separated.

Methyl-6-Hydroxymethylpiperonylate.

6-Hydroxymethylpiperonylic acid (1g) was dissolved in dry methanol (50ml) and cooled in ice-salt mixture to below 0°. Diazomethane[145] was bubbled through the solution and a bulky mass of white shining needles separated. They were

identified as 4,5-methylenedioxyphthalide.

6-Chloromethyl-3,4-methylenedioxybenzoylchloride.

(I) 6-Hydroxymethylpiperonylic acid (580mg) was refluxed for 2 hours with PCl_5 (800mg) and dry benzene (10ml). On cooling white shining needles of 4,5-methylenedioxyphthalide separated.

(II) 6-Hydroxymethylpiperonylic acid (300mg) was thoroughly mixed with PCl_5 (500mg) in a pestle and mortar with precautions against access of moisture; the mixture was transferred to a dry round-bottom flask and heated on an oil bath at 140-150° for 2 hours. 4,5-methylenedioxyphthalide condensed on the cooler part of the flask.

(III) 6-Hydroxymethylpiperonylic acid (300mg) was refluxed for 2 hours with PCl_5 (500mg) and thionyl chloride (5ml) using a calcium chloride tube to prevent entry of moisture. Evaporation of the excess of thionyl chloride under vacuum yielded a residue which was washed with a little benzene and identified as 4,5-methylenedioxyphthalide.

(IV) 6-Hydroxymethylpiperonylic acid (500mg) was refluxed for 3 hours with thionyl chloride (5ml) in an apparatus fitted with a calcium chloride tube. Excess of thionyl chloride was distilled off under vacuum and the residue was washed with benzene and identified as 4,5-methylenedioxyphthalide.

3,4-METHYLENEDIOXYPHENYLNITROMETHANE

Homopiperonyl alcohol was prepared by the reduction of piperonaldehyde with lithium aluminium hydride by the method of Davidson and Morston[146] and obtained as white needle crystals in 95% yield, m.p. 52-53°, literature m.p. 52-54°.

Homopiperonyl bromide was prepared from homopiperonyl alcohol by the method of Robinson and Robinson[144] as long white needles (from petroleum ether) m.p. 49°, yield 90%.

Attempted preparation of 3,4-methylenedioxyphenylnitromethane

Homopiperonyl bromide (2g) was dissolved in dry benzene (15ml) in 100 ml conical flask covered with carbon paper to prevent access of light and fitted with a calcium chloride tube to afford protection from moisture. Dry powdered silver nitrite (3g) was added and the mixture was shaken thoroughly for 10 minutes. Brown fumes of oxide of nitrogen were evolved, and dry nitrogen was passed in to remove the brown fumes. The flask was kept in the dark for one week with occasional shaking and passing in of dry nitrogen[74] from time to time. After one week the silver bromide which was washed with dry benzene, the washings being added to the filterate. The solvent evaporated at room temperature under vacuum, and the thick dark red oil

on sublimation gave piperonaldehyde in a poor yield. It was identified by infrared absorption and mixed m.p. 33-35°.

The experiment was repeated under various conditions and the results are recorded in table 4.

T A B L E 4

Homopiperonyl Bromide (gm)	Reagents	Temperature.	Time (Hrs)	Product.
1	Silvernitrite (1gm)	Room Temperature.	2	Piperonaldehyde.
3	Benzene(10ml) and silver nitrite (3gm)	" "	2	"
1	" "	" "	1	"
1	" "	" "	0.5	"
1	Ether(20ml)and Silver nitrite (1gm)	Refluxed	4	Piperonaldehyde and starting material
2	" "	Room Temperature	72	" "

Homopiperonyl nitrile was prepared from homopiperonyl bromide by the method of Braun and Wirz[147] as a golden yellow oil which crystallised from dilute alcohol in the form of needles m.p. 42-43°, literature m.p. 43-44°.

3,4-Methylenedioxyphenylnitromethane was prepared by the method reported in Organic Synthesis[98] for the preparation of phenylnitromethane. In a (50ml) round bottomed flask fitted with an efficient reflux condenser was placed absolute ethanol (10ml). Freshly cut metallic sodium (1g) was added rapidly and the flask was heated in an oil bath (100-110°) for 30 minutes. The sodium ethoxide began to precipitate and absolute ethanol (2ml) was added. The mixture was cooled to 0°c and a second portion of absolute ethanol (2ml) was poured on top of the solid cake. The reflux condenser was replaced by a stopper carrying a separatory funnel and a calcium chloride tube. An ice cold mixture of homopiperonyl nitrile (6.5g) and freshly prepared methyl nitrate[148] (5ml) was added dropwise with constant shaking, the temperature being maintained at about 4-8°. The reaction mixture was shaken intermittently for one hour and allowed to remain at 4-8° overnight. The grey sodium salt of the aci-nitro compound (5.1g) was filtered with suction, washed thoroughly with dry ether and air dried. The mother liquor and ether washings were combined and concentrated under reduced pressure to give more sodium salt (400mg).

Sodium hydroxide (5.5g) was dissolved in water (30ml) in a 100 ml beaker and heated to boiling. The air-dried crude sodium salt (5.5g) was added in small portion to the

boiling alkali. Boiling was continued until the evolution of ammonia ceased (3hours), hot water being added from time to time to keep the volume fairly constant. The hot alkaline solution was cooled to room temperature to give a waxy solid cake and the beaker and contents were kept in an ice-salt mixture provided with an efficient mechanical stirrer. Ice chips (10g) were added to the solid cake, and when the temperature was -5°, hydrochloric acid (16ml) was added dropwise with vigorous stirring, the temperature not being allowed to exceed -5°.

The cold solution was extracted with ether until the whole solid dissolved. The ether was washed with cold saturated sodium bicarbonate solution (50ml x 2) and water (50ml x 2) and dried (Na_2SO_4). Evaporation of the ether gave pale yellow 3,4-methylenedioxyphenylnitromethane. It was recrystallised from ethanol as pale yellow needles m.p. 55°, which were slowly soluble in N sodium hydroxide solution. The sodium bicarbonate extract on keeping overnight deposited more 3,4-methylenedioxyphenylnitromethane (400mg).

Found:	C, 53.29;	H, 4.09;	N, 7.20%
$C_8H_7NO_4$ requires.	C, 53.03;	H, 3.86;	N, 7.73%

3,4-Methylenedioxyphenylnitromethane was prepared from homopiperonyl bromide and sodium nitrite according to the method of Kornblum, Larson, Blackwood, Hooberry, Oliveto and Graham[85], for the preparation of phenylnitromethane. It was identical with that obtained *via* the nitrile.

6-BROMO-3,4-METHYLENEDIOXYPHENYLNITROMETHANE

6-Bromohomopiperonyl bromide was prepared from homopiperonyl alcohol by the method of Barthel and Alexander[102] as white needles m.p. 91-92°, literature m.p. 91-93°.

Attempted preparation of 6-bromo-3,4-methylenedioxyphenyl-nitromethane.

(I) 6-Bromohomopiperonyl bromide (300mg) was shaken for 15 minutes at room temperature with dimethylformamide (8ml) and silver nitrite (300mg). The mixture was filtered and the filtrate was cooled in ice and diluted with water (15ml) to give an oil which gradually crystallised. The crystals were filtered off, washed with a little cold water and recrystallised from hot ethanol (150mg) m.p. 93-95°.

Found: C, 42.7; H, 2.67; N, 0%

$C_9H_7O_4Br$. requires. C, 41.7; H, 2.7%

The compound was identical in infrared absorption, m.p. and mixed m.p. with an authentic sample of 6-bromohomopiperonyl formate.

(II) The experiment was repeated at -15° to -20° according to the method of Kornblum, Larson, Blackwood and Hoberry, Oliveto and Graham[92], 6-bromohomopiperonyl formate was obtained in 40% yield. Infrared absorption was identical with that of the product

obtained at room temperature.

(III) 6-Bromohomopiperonyl bromide (300mg) was dissolved in glacial acetic acid (5ml) and dry powdered silver nitrite (300mg) was added. The mixture was shaken for a few minutes and a precipitate was filtered off. The filtrate was cooled in ice and diluted with water (20ml) to give white fluffy needles which were washed with cold water and recrystallised from ethanol to give 6-bromohomopiperonyl acetate m.p. 80-82°, mixed m.p. 80-82°, identical with an authentic sample prepared from 6-bromohomopiperonyl bromide by the method of Barthell and Alexander[102].

The experiment was repeated under various conditions and the results are summarised in Table 5.

TABLE 5

6-Bromohomopiperonyl bromide (g)	Reagents	Temperature (C)	Time	Product
3	Benzene(30ml)and silver nitrite(3g)	Room Temperature	1week	6-Bromopiperonaldehyde
1	Silver nitrite(1g)	"	2hour	"
1	Benzene(10ml) and silver nitrite(1g)	"	2hour	"
1	" "	"	1hour	"
1	" "	"	0.5 "	6-bromopiperonaldehyde & 6-bromohomopiperonyl bromide.
1	Ether(20ml)and silver nitrite(1g)	50	4hours	6-bromopiperonaldehyde
1	" "	Room Temperature	72 "	"
2	Dimethylformamide (10ml)& silver nitrite (2g)	"	24 "	6-bromopiperonaldehyde & start.material
1	Benzene(10ml)& sodium nitrite(1g)	"	24 "	starting material.

6-Bromohomopiperonyl nitrile was prepared from 6-bromohomopiperonyl bromide by the method of Braun and Wirz[147] for homopiperonylnitrile as white shining needles m.p. 70-71°. Naik and Wheeler[78] gave m.p. 71-72°. In addition to 6-bromohomopiperonyl nitrile, white shining flakes were also obtained m.p. 184-185° which showed absorption at 2225cm^{-1}.

Found: C, 45.11; H, 2.69; N, 3.5, Br, 35.
Mol.wt.(Rast) 480.

$C_{17}H_{11}N\ Br_2\ O_4$ requires C, 45.03; H, 2.42; N, 3.09; Br, 35.32%
Mol.wt. 453.

6-Bromo-3,4-methylenedioxyphenylnitromethane was prepared according to the method adopted by Kornblum, Larson, Blackwood and Moobery, Oliveto and Graham[92] for the preparation of phenylnitromethane. 6-Bromohomopiperonyl bromide (10g) was dissolved in redistilled dimethylformamide (20ml) and the solution was poured into a stirred mixture of dried sodium nitrite (4g) and dried urea (6g) dissolved in dimethylformamide (100ml) maintained at -15° to -20°. After five hours, ice cold water (500ml) was added and the mixture was extracted with ether, the combined ether extracts were washed with water and dried (Na_2SO_4). On evaporation the ether gave white crystals which were recrystallised from ethanol to give white needles (4g) m.p.

88-89°, slowly soluble in N sodium hydroxide solution.

(II) It was also prepared from 6-bromohomopiperonyl nitrile according to the method described on page 148, in 75% yield, m.p. 88-89°. The infrared absorption spectrum was identical with that obtained by method (I); mixed m.p. 88-89°.

Found:	C, 37.27;	H, 2.82;	N, 5.72%
$C_8H_6O_4NBr$, requires:	C, 36.92;	H, 2.31;	N, 5.38%

ATTEMPTED PREPARATION OF α-(2-NITRO-4,5-METHYLENEDIOXYPHENYL) 2-NITROCINNAMIC ACID

6-Nitrohomopiperonylic acid was prepared from homopiperonylic acid by the method of Tiermann[109] in the form of pale yellow leaflets m.p. 187-188°; methyl ester m.p. 103-105°. Tiermann gave m.p. 187-189° and for the methyl ester Greene and Robinson[149] gave m.p. 103-105°.

Reactions with the methylester of 6-nitrohomopiperonylic acid.

(I) 6-Nitrohomopiperonylic acid methylester (1.2g) was heated on an oil bath at 100-110° for 10 hours with acetic anhydride (15ml) and o-nitrobenzaldehyde (0.8gm). After cooling the mixture to room temperature, pale yellow needles separated and these were filtered off, washed with cold ethanol, and identified as starting material by infrared absorption and mixed m.p.103-105°.

(II) 6-Nitrohomopiperonylic acid methyl ester (1.2g) was dissolved in methanol (50ml) and potassium methoxide (0.35gm). o-Nitrobenzaldehyde (0.80g) was added and the solution was shaken on a mechanical shaker for 2 hours at room temperature. The reaction mixture was diluted with water (50ml) and extracted with ether (50ml x 3). The combined ether was dried (Na_2SO_4) and evaporated *in vacuo* to yield a dark red oil which did not crystallise and it was not investigated further.

Reaction with the ethyl ester of 6-nitrohomopiperonylic acid.

By the method of Reissert[111], freshly cut metallic sodium (0.23g) was dissolved in absolute ethanol (20ml) and the mixture was cooled in ice water. 6-Nitrohomopiperonylic acid ethyl ester (1.27g) was added followed by o-nitrobenzaldehyde (0.8g). The red mixture was kept at 40° for 3 days with occasional shaking. The dark red mixture was cooled in ice water, acidified with dilute hydrochloric acid and the ethanol was evaporated under reduced pressure. The residue was extracted with ether and the ether layer was washed with water, dried (Na_2SO_4) and evaporated to give a thick dark red oil which did not crystallise and was not investigated further.

Reactions with the sodium salt of 6-nitrohomopiperonylic acid.

The sodium salt of 6-nitrohomopiperonylic acid (1.24g) was heated for 12 hours on an oil bath at 100-110° with acetic anhydride (40ml) and o-nitrobenzaldehyde (0.8g) according to the method of Shirai and Oda[150]. The dark brown reaction mixture was diluted with water (50ml) and warmed gently on a water bath with occasional shaking until the acetic anhydride was decomposed. The mixture was cooled in a freezing mixture overnight and the fine white needles (100mg) which separated, were filtered, washed with a little water and recrystallised from hot

ethanol. The mother liquor was evaporated to dryness to give a dark brown resinous solid, which was extracted with 5% ammonium hydroxide solution (50ml x 3). The basic solution was washed with ether and on acidification the solution gave dark brown micro-crystals (60mg)which were recrystallised from ethanol as white needles m.p. 240-246°.

Found: C, 56.08; H, 4.00; N, 7.04%

Calculated for $C_9H_7NO_4$: C,56.0; H, 3.62; N,7.24%

It was identified as o-nitrocinnamic acid by infrared absorption and mixed melting point (242-246°) with an authentic sample.

The experiment was repeated under various conditions and the results are summarised in Table 6.

T A B L E 6

Weight of material (g)	Reagents	Temperature (C)	Time	Results	Refs.
1.24	Acetic anhydride and o-nitrobenzaldehyde.	100-110	10 hrs.	o-nitrocinnamic acid	
1.24	" "	" "	8 Hrs.	" "	150.
1.24	" "	" "	4 Hrs.	" "	
1.24	" "	Room Temp.	3weeks	Starting material	
1.2 (Free acid)	Quinoline and o-nitrobenzaldehyde	"	3weeks	" "	151
1.2	Acetic anhydride o-nitrobenzaldehyde & triethylamine.	"		The compound decomposed	152

ATTEMPTED PREPARATION OF α-NITRO-6-BROMO-3,4-METHYLENEDIOXY-2′-NITROSTILBENE.

(I) 6-Bromo-3,4-methylenedioxyphenylnitromethane (0.52g) was heated for 10 hours on an oil bath at 100-110° with acetic anhydride (10ml) and o-nitrobenzaldehyde (0.35g). The mixture was cooled and 6-bromo-3,4-methylenedioxyphenylnitromethane separated as long pale yellow needles.

(II) 6-Bromo-3,4-methylenedioxyphenylnitromethane (0.52g) was heated for 12 hours on an oil bath at 100-110° with acetic anhydride (10ml), o-nitrobenzaldehyde (0.35g) and triethylamine (0.2ml). The dark red mixture was diluted with water (20ml) and warmed gently on a boiling water bath to decompose the acetic anhydride. The mixture was extracted with ether (50ml x 3) and the combined ether extract was washed with water, dried (Na_2SO_4) and evaporated to give pale yellow crystals (50mg). Sublimation yielded white crystals m.p. 200-202° identified as crude o-nitrocinnamic acid by infrared absorption.

(III) 6-Bromo-3,4-methylenedioxyphenylnitromethane (0.52g) was refluxed for 6 hours with absolute ethanol (20ml), sodium ethoxide (0.5g) and o-nitrobenzaldehyde (0.35g) according to the method of Fraser and Kon[113], and Nightingale, Erickson and Knight[114]. The dark brown

mixture was diluted with water (200ml) and the ethanol was evaporated under reduced pressure. The dark brown residue was extracted with ether and the ether extracts were dried (Na_2SO_4). The ether was evaporated to give a dark brown oil (200mg) which on sublimation gave 6-bromopiperonaldehyde identified by infrared absorption and mixed m.p. 127-128°.

(IV) 6-Bromo-3,4-methylenedioxyphenylnitromethane (0.52g) and o-nitrobenzaldehyde (0.35g) were dissolved in ethanol (20ml) and a few drops of ethylamine were added according to the method of Knoevengel and Walter[76]. White shining crystals (120mg) separated.

Found: C,39.72; H,4.23; N,8.82%

$C_{10}H_{13}O_4N_2Br$, requires: C,39.34; H,4.26; N,9.18%

These crystals were recrystallised from hot ethanol and 6-bromo-3,4-methylenedioxyphenylnitromethane was isolated, identified by infrared absorption and mixed m.p. 88-89°.

(V) The condensation experiment was attempted under different conditions and the results are summarised in Table 7.

TABLE 7

Experiment No.	Temperature (C)	Time (hrs)	Solvent	Catalyst	Results.
1	130	15	Acetic anhydride		Starting material
2	130	20	" "		Starting material
3	110	2	" "	Triethylamine[153]	o-nitrocinnamic acid
4	Room Temp.	24	" "	" "	o-nitrocinnamic acid
5	" "	1week	Ethanol	Sodium ethoxide[134]	6-Bromopiperonaldehyde
6	80	3 hrs	"	Triethylamine	crystalline compound A. m.p. 198-200°.
7	Room Temp.	10days	Pyridine.	" "	Compound A.
8	80	8 hrs	Ethanol.	n-butylamine[154]	Compound A.
9	80	5 hrs	Ethanol.	Sodamide[155]	Oily residue.
10	110	12hrs.	Pyridine		6-Bromopiperonaldehyde and a nitrile or isocyanide(unidentified)
11	85	8 hrs	Pyridine		" "
12	105	12hrs	"	Diethylamine[112]	" "
13	100	2 hrs	"	"	" "
14	110	12hrs	Quinoline		" "

continued:

TABLE 7

Experiment No.	Temperature (C)	Time (hrs)	Solvent	Catalyst	Results.
15.	105	12hrs.	Piperidine[156]		6-bromopiperonaldehyde

Compound A. Found: C, 46.18; H, 2.68; N, 4.43%

$C_{23}H_{14}O_8N_2Br$, requires: C, 45.4 ; H, 2.31; N, 4.62%

α-NITRO-6-BROMO-3,4-METHYLENEDIOXY-2′-NITROSTILBENE

6-Bromo-3,4-methylenedioxyphenylnitromethane (1.04g) and o-nitrobenzaldehyde (0.65g) were dissolved in ethanol (20ml) and one drop of ethylamine was added. The mixture was kept at room temperature for two weeks. The colour of the reaction mixture gradually changed from colourless to yellow and after 7 days some yellow crystalline material separated. Complete separation occurred after 14 days and the yellow crystals were filtered off and recrystallised from hot ethanol to give glistening yellow flakes (1.35g) yield 80%, m.p. 184-185°.

Found: C, 45.48; H, 2.23; N, 7.81%

$C_{15}H_9N_2O_6Br$, requires: C, 45.80; H, 2.29; N, 7.12%

α-NITRO-6-BROMO-3,4-METHYLENEDIOXY-2′-NITRO-6′-METHOXYSTILBENE

6-Bromo-3,4-methylenedioxyphenylnitromethane (2.6g) and 2-nitro-6-methoxybenzaldehyde (1.9g) were dissolved in ethanol (40ml), one drop of ethylamine was added and the mixture was kept at room temperature for two weeks. The solvent was evaporated at room temperature under reduced pressure to yield an oily residue which was cooled in an ice bath. Yellow crystals (3.6gm) were filtered off and recrystallised from hot ethanol to give yellow glistening needles m.p.200°.

Found: C,45.73; H,2.31; N,6.78; Br,19.1%

$C_{16}H_{11}N_2O_7Br$, requires: C,45.39; H,2.6; N,6.62; Br,18.91%

α-NITRO-3,4-METHYLENEDIOXY-2′-NITROSTILBENE

3,4-Methylenedioxyphenylnitromethane (1.81g) and o-nitrobenzaldehyde (1.6g) were treated as described for α-nitro-6-bromo-3,4-methylenedioxy-2′-nitro-6′-methoxystilbene. The crystals were recrystallised from ethanol as bright red needles m.p. 114-115°, yield 80%.

Found: C, 57.71; H, 3.88; N, 9.32%

$C_{15}H_{10}O_6N_2$ requires: C, 57.32; H, 3.18; N, 8.91%

α-NITRO-3,4-METHYLENEDIOXY-2′-NITRO-6′-METHOXYSTILBENE

3,4-Methylenedioxyphenylnitromethane (0.382g) and 2-nitro-6-methoxybenzaldehyde (0.382g) were treated as described for α-nitro-6-bromo-3,4-methylenedioxy-2′-nitro-6′-methoxystilbene. The crystals were recrystallised from ethanol to give bright yellow flakes m.p. 162°, yield 80%

Found: C, 55.93; H, 3.46; N, 8.27%

$C_{16}H_{12}N_2O_7$ requires: C, 55.81; H, 3.48; N, 8.14%

α-NITRO-6-BROMO-3,4-METHYLENEDIOXY-6′-METHOXYSTILBENE

6-Bromo-3,4-methylenedioxyphenylnitromethane (1.3g) and o-methoxybenzaldehyde (0.69g) were treated as described for α-nitro-6-bromo-3,4-methylenedioxy-2′-nitro-6′-methoxystilbene. The crystals were recrystallised from ethanol as bright yellow flakes m.p. 153-154°.

Found: C,50.86; H,3.0; N, 3.64.

$C_{16}H_{10}NO_5Br$ requires: C,50.79; H,3.17; N,3.7; Br,21.16%

α-NITRO-6-BROMO-3,4-METHYLENEDIOXYSTILBENE.

6-Bromo-3,4-methylenedioxyphenylnitromethane (0.52g) and benzaldehyde (0.21g) were treated as described for α-nitro-6-bromo-3,4-methylenedioxy-2′-nitro-6′-methoxystilbene. The crystals were recrystallised as pale yellow flakes m.p. 119-120°.

Found:	C, 51.41;	H, 2.84;	N, 3.65%
$C_{15}H_{10}NO_4Br$, requires:	C, 51.43;	H, 2.87;	N, 4.02%

α-(3,4-Methylenedioxy-6-bromophenyl) 2-nitrocinnamic acid. was prepared by condensation of o-nitrobenzaldehyde and 6-bromohomopiperonylic acid by the method of Pailer and Schleppnik[152], as pink crystals m.p. 237-238°, literature m.p. 238-9°. It was decarboxylated by heating with quinoline and copper to give a poor yield of yellow crystals of 6-bromo-3,4-methylenedioxy-2′-nitrostilbene m.p. 157-163°.

α-(3,4-Methylenedioxyphenyl) 2-nitrocinnamic acid was prepared by condensation of o-nitrobenzaldehyde and homopiperonylic acid by the method of Pailer and Schleppnik[152] as yellow flakes m.p. 226-228°, literature m.p. 226-8°. It was decarboxylated by heating with quinoline and copper to give yellow crystal of 3,4-methylenedioxy-2′-nitrostilbene m.p. 106-108°.

REDUCTION OF NITRO-COMPOUNDS

Attempted preparation of α-nitro-6-bromo-3,4-methylenedioxy-2′-aminostilbene.

(I) α-Nitro-6-bromo-3,4-methylenedioxy-2′-nitrostilbene (0.2g) was dissolved in ethanol (30ml), boiled on a water bath and ammonium sulphide solution (1ml 10%W/V) was added dropwise. The mixture was further boiled for 5 minutes and when cooled to room temperature, fine yellow needles (50mg) were deposited. They were recrystallised from ethanol m.p. 164-165°, and concentration of the mother liquor deposited a further 10mg of the crystals (yield 33%).

Found: C,51.57; H,2.97; N,4.22; Br,23.5%

$C_{15}H_{10}N\ O_4Br$, requires: C,51.43; H,2.87; N,4.02; Br,23.0%

(II) The experiment was repeated using double the theoretical amount of ammonium sulphide to give the same crystalline material m.p. 164-165° that was isolated above.

(III) α-Nitro-6-bromo-3,4-methylenedioxy-2′-nitrostilbene (0.2g) was dissolved in ethanol (80ml), and concentrated ammonia solution (5ml) was added and the mixture was cooled in ice bath to below 0C. Hydrogen sulphide[120] was passed through the solution for 15 minutes and the reaction mixture was boiled for

5 minutes to remove excess of ammonia and hydrogen sulphide. The solution was cooled to room temperature to give crystals m.p. 164-165°, identical with those isolated in experiment (I) and (II) above.

The product isolated from the reduction of compound was further reduced with zinc and dilute hydrochloric acid to yield white needles m.p. 180°.

(IV) α-Nitro-6-bromo-3,4-methylenedioxy-2′-nitrostilbene (0.197g) was hydrogenated in glacial acetic acid (60ml) at platinum oxide catalyst (20mg). Hydrogen uptake was complete after 20 minutes with the absorption of 37 ml (at N.T.P.) sufficient for the reduction of one nitro group and for the reduction of catalyst. The solution was filtered and concentrated at room temperature under reduced pressure to yield a mixture of four components as found by thin layer chromatography using petroleum ether/ether (1:1) as solvent. Detection of the components was carried out by spraying with sulphuric acid (50% v/v) and heating carefully on a hot plate.

(V) α-Nitro-6-bromo-3,4-methylenedioxy-2′-nitrostilbene (0.393g) was mixed with ethanol (5ml). The mixture was boiled on a water bath, two drops of concentrated hydrochloric acid were added followed by iron powder

(170mg) in small portion with continuous stirring and boiling. After the complete addition of the iron, the mixture was refluxed for two hours. Ethanol (50ml) was added and the dark brown solution was filtered. The solvent was evaporated at room temperature under reduced pressure to yield a dark brown resinous mass, which on sublimation gave a mixture of starting material and a nitrile (unidentified) and it was not investigated further.

(VI) α(-Nitro-6-bromo-3,4-methylenedioxy-2′-nitrostilbene (0.2g) was dissolved in ethanol (30ml) and concentrated ammonia solution (5ml) added. The mixture was refluxed on water bath for 4 hours and the solvent was evaporated under reduced pressure to yield pale yellow crystals m.p. 198-200°. Infrared absorption spectra showed a similarity with compound A. (Table 7, page 161).

The experiment was repeated under various conditions and the results are summarised in Table 8.

TABLE 8

Experiment No.	Solvent	Reagents	Temperature (C)	Time	Results.
1.	Ether	Lithium aluminium hydride	40	1hour	Pale brown resin
2.	"	" "	Room Temp.	3mts.	White crystals m.p.165-167° (not investigated)
3.	Ethanol and glacial acetic acid	Titanus chloride 158	" "		Starting material
4.	Ethanol.	Ammonium hydroxide 159 and ferrous sulphate	Water bath	20mts.	Dark resinous material.

Attempted Preparation of α-nitro-6-bromo-3,4-methylenedioxy-2′-Amino-6′-methoxystilbene.

(I) α-Nitro-6-bromo-3,4-methylenedioxy-2′-nitro-6′-methoxystilbene (0.423g) was dissolved in ethanol (50ml), boiled on a water bath and ammonium sulphide solution (2ml 10% W/V) was added dropwise. The solution was boiled for a further 5 minutes and the mixture was cooled to room temperature to yield yellow needles of starting material, confirmed by infrared absorption and mixed m.p.

(II) The above experiment was repeated using double the theoretical amount of ammonium sulphide solution. Pale yellow crystals were recovered which could not be purified.

Attempted Preparation of α-nitro-3,4-methylenedioxy-2′-aminostilbene.

(I) α-Nitro-3,4-methylenedioxy-2′-nitrostilbene (0.314g) was dissolved in ethanol (50ml), the solution was boiled on a water bath and ammonium sulphide solution (2ml 10% W/V) was added dropwise. The mixture was boiled for 5 minutes and cooled to room temperature to yield yellow crystals (0.2g). These were recrystallised from ethanol as glistening yellow needles m.p. 110-111°.

Found:	C, 65.78;	H, 4.34;	N, 5.0%
$C_{15}H_{11}N\ O_4$ requires:	C, 66.91;	H, 4.08;	N, 5.2%

(II) α-Nitro-3,4-methylenedioxy-2′-nitrostilbene (0.157g) was hydrogenated in ethanol (50ml) at platinum oxide catalyst (20mg). Hydrogen uptake was complete after 20 minutes with the absorption of 37ml at (N.T.P.) sufficient for reduction of one nitro group and reduction of catalyst. The solution was filtered and evaporated at room temperature under reduced pressure to yield pale yellow crystals which were a mixture of four components, found by thin layer chromatography using the system previously described (page 167).

Thin layer chromatography

The products obtained by reduction with ammonium sulphide from α-nitro-6-bromo-3,4-methylenedioxy-2′-nitrostilbene and α-nitro-3,4-methylenedioxy-2′-nitrostilbene, 6-bromo-3,4-methylenedioxy-2′-nitrostilbene, 3,4-methylenedioxy-2′-nitrostilbene and α-nitro-6-bromo-3,4-methylendioxystilbene were chromatographed on silica gel plate using petroleum ether/diethyl ether (1:1) as solvent. Detection of the compounds was carried out by spraying with sulphuric acid (50% V/V) and heating carefully on a hot plate. The results are recorded in Table

T A B L E 9

Constituents.	Rf Value.
1. Reduced product from α-nitro-6-bromo-3,4-methylenedioxy-2′-nitrostilbene.	0.71.
2. 6-bromo-3,4-methylenedioxy-2′-nitrostilbene.	0.70.
3. Reduced product from α-nitro-3,4-methylene-dioxy-2′-nitrostilbene.	0.65.
4. 3,4-methylenedioxy-2′-nitrostilbene.	0.62.
5. α-nitro-6-bromo-3,4-methylenedioxystilbene.	0.69.

ATTEMPTED PREPARATION OF 6-CYANO-3,4-METHYLENEDIOXY-2′-NITROSTILBENE

6-Bromo-3,4-methylenedioxy-2′-nitrostilbene (0.173g) was refluxed for 12 hours in dry pyridine (10ml) with silver cyanide (0.2g) and cuprous cyanide (0.1g) using a small amount of anhydrous copper sulphate as activator. The mixture was diluted with water (20ml) and extracted with ether (100ml x 3). The combined ether layers were extracted with cold dilute hydrochloric acid until no pyridine was left in ether. The ether was dried (Na_2SO_4) and evaporated to give starting material confirmed by infrared absorption and mixed melting point.

6-CYANO-3,4-METHYLENEDIOXY-2′-NITROSTILBENE

6-Bromo-3,4-methylenedioxy-2′-nitrostilbene (0.346g) was refluxed for 4 hours in dry quinoline[160] (10ml) with silver cyanide (0.3g), cuprous cyanide (0.2g) and a small amount of anhydrous copper sulphate. The mixture was diluted with water (20ml) and extracted with ether (100ml x 3). The combined ether extracts were extracted with cold dilute hydrochloric acid until no quinoline was left in the ether. The ether was dried (Na_2SO_4) and evaporated to yield greenish yellow needles (0.08g) which on recrystallisation from ethanol melted at 191-192°.

Infrared absorption at 2225cm^{-1} (nitrile).

Found: C, 63.84; H, 3.52; N, 9.0%

$C_{16}H_{10}N_2O_4$ requires: C, 65.3; H, 3.4; N, 9.52%

ATTEMPTED PREPARATION OF 6-CARBOXY-3,4-METHYLENEDIOXY-2′-NITROSTILBENE

(I) Dry magnesium (50mg) was covered with dry ether (20ml) and 6-bromo-3,4-methylenedioxy-2′-nitrostilbene (30mg) and a crystal of iodine were added. The mixture was refluxed gently with continuous stirring. The rest of the 6-bromo-3,4-methylenedioxy-2′-nitrostilbene (0.47g) dissolved in ether (200ml) was added dropwise so that the reaction mixture refluxed gently. After the addition was complete, the mixture was refluxed for 6 hours and cooled in an ice-salt mixture below 0^{o}. Dry carbon dioxide was passed through the solution for 20 minutes and dilute sulphuric acid was added, the temperature being maintained below 0^{o}. The ether layer was separated and the aqueous layer was further extracted with ether. The bulked ether layers were washed with water, dried (Na_2SO_4) and evaporated to yield starting material, confirmed by infrared absorption and mixed melting point.

(II) The experiment was repeated using a reflux time of 12 hours, but starting material was isolated.

(III) The above experiment was repeated with lithium using a reflux time of 24 hours, but starting material was isolated.

(IV) The experiment was repeated by the entrainment method[136]

using magnesium methyl iodide as an activator. The solution became highly fluorescent and on evaporation of the ether pale yellowish brown crystals (non-fluorescent) were isolated. They could not be recrystallised.

ATTEMPTED PREPARATION OF α-NITRO-6-BROMO-3,4-METHYLENEDIOXY-2′-ACETYLAMINOSTILBENE

(I) 6-Bromo-3,4-methylenedioxyphenylnitromethane (1.3g) and o-acetylaminobenzaldehyde[161,162] (0.82g) were dissolved in ethanol (50ml) and a drop of ethylamine was added. The mixture was kept at room temperature for 14 days. Starting material was recovered from the reaction mixture.

(II) 6 Bromo-3,4-methylenedioxyphenylnitromethane (1.3g) and o-acetylaminobenzaldehyde (0.82g) were dissolved in ethanol (50ml) and added dropwise to an ice cold sodium hydroxide solution as described by Worrall[135], but starting material was recovered.

ATTEMPTED PREPARATION OF α-NITRO-3,4-METHYLENEDIOXY-2′-ACETYLAMINOSTILBENE

(I) 3,4-Methylenedioxyphenylnitromethane (1.81g) and o-acetylaminobenzaldehyde (1.53g) were dissolved in ethanol (60ml) and one drop of ethylamine was added. The mixture was kept at room temperature for 14 days, but starting material was recovered.

(II) The above experiment was repeated according to the method of Worrall[135], but starting material was recovered.

IRRADIATION

α-Nitro-6-bromo-3,4-methylenedioxy-6′-methoxystilbene and 6-bromo-3,4-methylenedioxy-2′-nitrostilbene were irradiated on a micro-scale in cyclohexane for 2½ hours according to the method of Mallory, Wood and Gordon[139] for the preparation of phenanthrene derivatives. Both the compounds were decomposed. The experiment was repeated by irradiation for 5,10,15,20, and 30 minutes but no absorption characteristic of the phenanthrene nucleus was observed.

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APPENDIX

DISCUSSION

ARISTOLOCHIA GOLDIEANA

Rhizomes

A mixture of sterols was isolated from the light petroleum extract and infrared examination of the crystals indicated the presence of β-sitosterol. No satisfactory derivatives were obtained.

The fatty acids in the extract were obtained as the methyl esters but in too small a quantity for fractional distillation. The esters were, however identified by gas chromatography as myristate, palmitate, oleate, stearate and linoleate.

From the ethanolic extract of the defatted rhizomes were obtained 5 mg of yellow crystals m.p. 265° (decomp.). The infrared absorption curve of which showed the presence of a carboxyl and a nitro-group. The crystals cannot be impure aristolochic acid, however, because the absorption curve of the latter has bands at about 755, 825, 1080, and 1270cm^{-1} which are absent from absorption curve of the former. It would be of interest to re-examine this species when more material becomes available.

Fibrous Roots

Aristolochic acid only was sought for in the ethanolic extract but none was found.

EXPERIMENTAL

ARISTOLOCHIA GOLDIANA

Light petroleum soluble extract of Rhizomes.

Isolation of Sterols.

The rhizomes (200gm) were reduced to coarse powder and defatted with petroleum ether (40-60°). Evaporation of the solvent gave a thick dark brown oil (2.14gm) with a pleasant smell. On standing overnight white crystalline material (20mg) was deposited. The crystalline solid was filtered, washed with a little cold ethanol and recrystallised from ethanol to give white shining crystals some of which melted at 138-140° and the remainder at 148-150°. Infrared absorption was similar to that of β-sitosterol. The filtrate was reserved for saponification and isolation of acids.

Preparation of Sterol acetate

The crystals (10mg) were dissolved in dry pyridine (1ml) and two drops of acetic anhydride were added, and the mixture was heated over a water bath for two hours. The solution was cooled in a refrigerator and the crystals which separated were filtered off, washed with cold ethanol and recrystallised from ethanol. The crystals softened at 75° and melted completely at 132°.

Isolation of fatty acids.

The oil (page 189) was saponified with ethanolic

potassium hydroxide (0.5gm of potassium hydroxide in 20ml of ethanol) till it gave a clear solution in water (2 hours). Ethanol was evaporated under reduced pressure, and water was added to maintain the volume. The soap solution was diluted with water, extracted with petroleum ether (40ml x 3), the petroleum ether layers were dried (Na_2SO_4) and the solvent was evaporated to give a light yellow oil of pleasant odour (220mg) containing some waxy crystalline material. It was not investigated further.

The aqueous solution was extracted with ether (40ml x 3), the combined ether extracts were dried (Na_2SO_4) and on evaporation, a brown semi crystalline mass (70mg) was obtained but it was not investigated further. The aqueous solution was acidified with dilute hydrochloric acid and the precipitated oil was extracted with petroleum ether. The dried ether layer was evaporated to give the fatty acids as a dark brown semi-solid (900mg). The fatty acids were refluxed for two hours on a water bath with methanol (10ml) and two drops of sulphuric acid. The solution was cooled, diluted with brine and extracted with petroleum ether (30ml x 3). The combined petroleum ether extracts were washed with brine, water, saturated solution of sodium bicarbonate and again with water and dried (Na_2SO_4). Evaporation of the solvent yielded the methyl esters as a dark brown semi-solid (700mg) which was distilled *in vacuo*

to give a very pale yellow oil. Examination of the oil by gas chromatography as described on page 52 showed the presence of methyl myristate and linoleate (minor components) and methyl palmitate, oleate and stearate (major components).

Ethanol extract of rhizomes

Isolation of a nitro-compound

The dried powder previously defatted with petroleum ether was extracted in Soxhlet extractor with ethanol for 10 hours. Concentration of the extract gave a viscous dark oil, which deposited waxy crystalline material. Recrystallisation of this material from ethanol after decolorisation with charcoal gave pale white waxy crystals (8mg) not sufficiently pure for investigation. The oil was treated as described under A. indica (page 134) to give a yellow oil (206mg) with some yellow crystals (8mg) m.p. 265° (decomp.). Infrared absorption at 1520cm^{-1} (NO_2).

Chromatography of nitro-compound

The crystals were chromatographed on Whatman No.1 paper with (4:1) ethanol 5% formic acid as solvent and aristolochic acid as control. Both gave a yellow spot under ordinary light having the same R_F value (0.90) but the sample showed considerable tailing and fluoresced under ultraviolet light.

Reduction of Nitro-compound.

The crystals were dissolved in glacial acetic acid (5ml) and a small amount of zinc dust was added. The mixture was warmed over a water bath for 30 minutes to give a yellow highly fluorescent solution.

Extraction of fibrous roots

The dry coarse powder (62g) was extracted with petroleum ether for 4 hours and on evaporation of the solvent, a yellow-brown semi-solid residue (0.515g) was obtained.

The defatted dried marc was extracted with hot ethanol for 8 hours. No aristolochic acid was obtained on the evaporation of the solvent and the residue was a yellow oil (0.157gm).

DISCUSSION

ARISTOLOCHIA INDICA

A small quantity of the aerial parts of the plant was available and it was examined for the presence of aristolochic acid. None was found.

EXPERIMENTAL

ARISTOLOCHIA INDICA

Dry coarsely powdered aerial parts (26gm) of A. Indica were defatted with petroleum ether. Evaporation of the solvent gave a pale yellow viscous oil (0.66gm) with a pleasant aromatic smell. The dry defatted powder was extracted with hot ethanol, and evaporation of the solvent did not give any aristolochic acid but only a pale yellow oil (0.052gm).

S U M M A R Y

SUMMARY

The thesis is presented in two parts with one appendix.

Part I was prompted by a report that the starch isolated from the seeds of Phalaris Canariensis possessed hypotensive activity. A review of the literature on Phalaris species is presented and the isolation of the starch and its extracts is described. No hypotensive activity of the starch or its extracts was found.

Chemical examination of aqueous extracts of the starch showed the presence of choline, glucose, maltose, azelaic acid and aminoacids. Ion exchange and paper chromatography were used to identify serine, α-alanine, phenylalanine, leucine, proline, aspartic acid, valine and aminobutyric acid.

The light petroleum soluble matter of the powdered seeds was examined and a mixture of sterols was isolated. β - sitosterol was identified in the mixture by conversion of the sterols to the methyl ethers and examination of the ethers by gas chromatography; two other components in the mixture were not identified. It is also shown that the "β - sitosterol" isolated from Aristolochia reticulata consisted of two components one of which is β - sitosterol. The fatty acids in the extract were identified as myristic, palmitic, stearic, oleic and linoleic acids, and glycerol was also isolated.

Part II has as its main theme the attempted synthesis of aristolochic acid. A review of the degradation work carried out on aristolochic acids is given followed by an account of the pharmacological activity of extracts and compounds isolated from Aristolochia species.

After a report on the synthesis of derivatives of aristolochic acid, in which the benzoate of 8 - methoxy -3,4-methylendioxy-10-nitro-1-phenanthroic lactam was obtained as the only new product, a route to aristolochic acid is proposed. This was based on the Pschorr Synthesis, as one of the required intermediates, 2-nitro-6-methoxybenzaldehyde was known. The attempted preparation of the other intermediate, 6-carboxy-3,4-methylenedioxyphenylnitromethane, is discussed and the structure for a new compound obtained during this work is suggested. The lack of success in this stage turned attention to the synthesis of 3,4-methylenedioxyphenylnitromethane and the corresponding 6-bromo-compound and both compounds were obtained by two routes. One of these involved the use of metal nitrites and the conditions used and reactions taking place are considered in some detail.

The condensation of the nitro-compound with benzaldehyde and substituted benzaldehydes was investigated and the following compounds were prepared.

α-nitro-6-bromo-3,4-methylenedioxy-2'-nitro-6'-methoxystilbene.

α-nitro-3,4-methylenedioxy-2'-nitro-6'-methoxystilbene.

α-nitro-6-bromo-3,4-methylenedioxy-6'-methoxystilbene

α-nitro-6-bromo-3,4-methylenedioxy-2'-nitrostilbene.

α-nitro-3,4-methylenedioxy-2'-nitrostilbene.

Side reactions occuring during the condensation are discussed and tentative structures advanced for two of the products. Attempted condensation of acetylaminobenzaldehyde with the substituted phenylnitromethanes met with no success. Selective reduction of α-nitro-6-bromo-3,4-methylenedioxy-2'-nitrostilbene and α-nitro-3,4-methylenedioxy-2'-nitrostilbene by ammonium sulphide caused the elimination of one nitro-group from each compound and evidence is presented to show that the products were 6-bromo-3,4-methylenedioxy-2'-nitrostilbene and 3,4-methylenedioxy-2'-nitrostilbene respectively. The reduction of the nitro-compounds was also attempted by a variety of reducing agents, but no satisfactory product was isolated. In order to avoid selective reduction of a nitro-group irradiation of α-nitro-6-bromo-3,4-methylenedioxy-6'-methoxystilbene and 6-bromo-3,4-methylenedioxy-2'-nitrostilbene was attempted on the micro-scale, but ultraviolet absorption evidence indicated that the phenanthrene nucleus was not formed.

Part II concludes with an assessment of the results obtained and a scheme for further work is proposed.

The appendix presents the results of an examination of extracts of small quantities of the roots and rhizomes of A. goldieana and of the aerial parts of A. indica. No aristolochic acid was isolated from the extracts.