Thesis

Selective Fermentation in Relation to the Resolution of Optically Active Compounds.

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By

Stotherd Thomas Richard Smith Mitchell, B.Sc.

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

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SELECTIVE FERMENTATION IN RELATION TO THE RESOLUTION OF OPTICALLY ACTIVE COMPOUNDS.

SELECTIVE FERMENTATION IN RELATION TO THE RESOLUTION OF OPTICALLY ACTIVE COMPOUNDS.

Selective Assimilation by Micro-organisms

Discoveries not unfrequently result from chance observations made in the pursuit of some quite different line of research. It was so when Pasteur (Comp. Rend. 1858 46 615) noticed that some of his tartrate solutions had commenced to undergo fermentation during warm weather. Commercial solutions of calcium tartrate had sometimes been known to behave in this way, but the observation set Pasteur wondering how micro-organisms would act on the two optical isomers of a salt of racemic acid. To test this he dissolved a quantity of ammonium racemate in water, added some nutritive substances and a small amount of a spontaneously fermented ammonium tartrate solution. He followed the course of the fermentation by means of the polarimeter, and found that the initially inactive liquid gradually developed a laevo rotation which increased to a maximum value. The dextro salt had thus been destroyed in preference to the laevo, and a quantity of l-ammonium tartrate was obtained from the solution. Unfortunately Pasteur does not describe the organism which was responsible for the fermentation , but it was probably a species of Penicillium, for two years afterwards

(Comp. Rend. 1860 51 298) he obtained the same result by the use of Penicillium glaucum.

Later it was shown that this mould, and several other varieties as well, could be used to obtain active forms from a number of racemic compounds. A list (with references) of the substances examined up to 1895 in this way is given in a paper by Winther (Ber. 1895 28 3022). The following active forms have been obtained from the corresponding racemic substances. It is interesting to note that in some cases it is the dextro form, and in others the laevo which is destroyed.

l-Tartaric acid (Pasteur)

d-tartaric acid (Lewkowitsch)

l-glyceric acid (Lewkowitsch)

d-glyceric acid (Frankland and Frew)

d-lactic acid (Lewkowitsch),(Linossier),and (Frankland and MacGregor)

d-ethoxysuccinic acid (Purdie and Walker)

l-aspartic acid (Engel)

l-glutaminic acid (Schulze and Bosshard) and (Menozzi and Appiani)

d-leucine (Schulze and Likiernik)

l-α-propylene glycol (Le Bel)

l-methyl-ethyl-carbinol (Combes and Le Bel)

l-methyl-N-propyl-carbinol (Le Bel)

l-methyl-butyl-carbinol (Combes and Le Bel)

d-ethyl-propyl-carbinol (Combes and Le Bel)

d-methyl-N-amyl-carbinol (Le Bel)

d-methyl-ethyl-carbin-carbinol (Le Bel)

d-mandelic acid (Lewkowitsch)

l-mandelic acid (Lewkowitsch)

d-cinnamic acid dichloride (Stavenhagen and Finkenbeiner)

l-isobutyl-propyl-ethyl-methyl-ammonium chloride (Le Bel)

Unfortunately, however, much of this work was carried out without any precautions being taken to ensure that only one species of micro-organism was present.

In the course of some work on the selection of organic nutritive substances for fungi, Pfeffer (Jarb. Wis. Botanik. 1895 221) repeated Pasteur's racemic acid experiment with several pure cultures, and found that both the dextro and laevo forms were attacked. In some cases the dextro isomer was destroyed more rapidly than the laevo, and in others the rates of decomposition appeared to be identical.

McKenzie and Harden (J.C.S. 1903 <u>83</u> 424) extended this work with pure cultures of Penicillium glaucum, Aspergillus niger and Aspergillus griseus to a number of racemic

acids. The type of rotation of the product after the growth of the mould is given in the following table.

Acid	P.g.	A.n.	A.gr.
Racemic	l	l	l
Dimethoxy succinic	l	l	l
Lactic	l	l	l
α-Aminopropionic	l	l	l
α-Ethoxypropionic	l	i	d
α-Propoxypropionic	l	-	-
α-Hydroxybutyric	l	l	l
β-Hydroxybutyric	l	l	d
Glyceric	l	l	l
Malic	l	-	-
Methoxysuccinic	l	-	-
Ethoxysuccinic	l	-	-
Propoxysuccinic	i	-	-
Mandelic	l	l	d
Methoxyphenylacetic	l	-	-
Ethoxyphenylacetic	l	-	d
Propoxyphenylacetic	l	l	-

The following conclusions were arrived at by these authors. " In the action of pure cultures of P.g., A.n. and A. gr. on salts of inactive acids our experience in most cases tends to show that the mode of action is such that the mould attacts the one active isomeride more readily than the other, and that the extent of the resolution depends solely upon the difference of the rate of attack. For obtaining the pure active isomeride the method is unsuitable."

From time to time other records of the action of micro-organisms on racemic bodies have been published. A note-worthy paper is that by Pringsheim who worked with leucine and glutamic acid. (Zeit. Physiol. Chem. 1910 65 96) In all he employed twenty-one different species of fungi. About half of these attacked the dextro and laevo forms at practically the same rate. The others showed preferential action on one isomer which proved in all cases to be the form which occurred naturally. Also Neuberg has cited some additional examples of bacteria which have preferential action, but the conclusions of McKenzie and Harden quoted above may be applied to moulds generally, and to bacteria as well. At least half of the racemic compound is destroyed in each case, and the isolation of the remaining active substance is often troublesome since dilute solutions have to be employed.

The yeasts are not so catholic in their tastes as the

moulds and bacteria, but they have been used very successfully for resolution purposes in a limited number of cases.

By the aid of beer yeast Fischer has prepared the following from racemic sugars.

l-Glucose (Fischer, Ber. 23 2620)

l-Mannose (Fischer, Ber. 23 382)

l-Galactose (Fischer and Hertz Ber. 25 1295)

l-Fructose (Fischer, Ber. 23 389, & 27 2031)

Ehrlich (Biochem. Zeit. 1906 1 8) found that yeast (in presence of sucrose solution) attacked the dextro and laevo components of externally compensated mixtures of alanine, leucine and α-amino-isovaleric acid at different rates. l-alanine, d-leucine and l-α-amino-isovaleric acid could be readily separated from the product in a 65-70% yield.

Buchner (Ber. 1897 30 117) has shown that a clear juice can be separated from the yeast cells by crushing and grinding with sand. From this liquid a ferment zymase was obtained which was capable of decomposing hexoses in the same way as yeast. Following the suggestion of Kühne such preparations are known as enzymes.

Selective Hydrolysis by Enzymes

The use of hydrolytic enzymes avoids the destruction of one of the active isomers, and both dextro and laevo forms (in varying degrees of optical purity) can often be isolated. The following cases have been investigated.

Maltase

Besides the sucroclastic enzyme zymase, yeast contains the hydrolytic enzyme maltase. Fischer (Ber. 1898 26 69) found that maltase would hydrolyse what he called α-methyl d-glucoside, but not α-methyl l-glucoside. The enzyme, however, had no action on the β-methyl glucosides. Emulsin (see page 10) the enzyme found in almonds, on the other hand hydrolysed β-methyl d-glucoside, but not β-methyl l-glucoside and had no action on the α-methyl glucosides. This has been found to be true for other alkyl glucosides as well.

Trypsin

Fischer and Bergell (Ber. 1903 36 2603) have shown that when carbethoxy glycyl dl-leucine is hydrolysed with trypsin, the carbethoxy l-leucine is attacked in preference to the corresponding compound of d-leucine. Quantitative measurements were not made, however, so it is not possible to say how completely the dl-leucine was separated into its antipodes.

Later Fischer and his collaborators found that many peptides are selectively attacked by trypsin. In all cases the products of hydrolysis are amino acids which are found in natural proteins. For example it was found (Fischer and Abderhalden, Zeit. Physiol. Chem. 1907 51 264)

Hydrolysable	Not Hydrolysable
d-Alanyl-d-alanine	d-Alanyl-l-alanine
d-Alanyl-l-leucine	l-Alanyl-d-alanine
l-leucyl-l-leucine	l-Leucyl-glycine
	l-Leucyl-d-leucine
l-Leucyl-d-glutaminic acid	d-Leucyl-l-leucine

Warburg (Ber. 1905 38 187) acted on the synthetic ethyl ester of leucine with crude pancreatin. The d- ester was not attacked, but the l- ester gave l-leucine. Later (Zeit. Physiol. 1906 48 205) he worked with pancreatin from which he had removed the lipase. Starting with n-propyl dl-leucine ester he obtained l-leucine, the ester of d-leucine being unattacked. In one experiment he recovered 70% of the l-leucine of $[\alpha]_D$ = +15 in 20% hydrochloric acid. In another, he obtained 70% of the l-leucine of $[\alpha]_D$ = +15·5 in 20% hydrochloric acid. For the pure substance $[\alpha]_D$ = +15·6 under the same conditions. He also

recovered the d-leucine from the unchanged ester, and it had $[\alpha]_D$ = - 12·4, so that there had been some unconverted l- ester present.

Lipase

Dakin (J. Physiol. 1904 30 253) studied the selective action of lipase on esters of mandelic acid. He started with an optically inactive mixture of the two methyl mandelates, and allowed the enzyme to partially hydrolyse it. When the mandelic acid liberated was separated off and examined, it was found to be dextro rotatory. If the hydrolysis was allowed to go to completion, however, the acid set free was inactive. He extended his experiment to the ethyl, iso-amyl and benzyl esters, and found that in each case the dextro component was hydrolysed more rapidly than the laevo one. The results for the ethyl mandelates are the most striking and are quoted on next page. He states that the results obtained are not sufficiently accurate to warrant a direct calculation of the relative rates of hydrolysis, but " It may be safely concluded that the ratio is not greater than 0·5."

For the pure acid $[\alpha]_D^{20}$ = 156

% Hydrolysed	$\frac{100a_D}{l}$	Conc. of mandelic acid soln	$[a]_D^{20}$	% d-mandelic acid
5·2	+ 11	·1844	+ 59·7	38·3
16·9	+ 63	1·337	+ 47·1	30·2
32·4	+ 71	1·854	+ 38·3	24·6
43·2	+ 90	2·553	+ 35·3	22·6
75·6	+ 34	1·490	+ 22·8	14·6
81·0	+ 41	1·945	+ 21·1	13·5
93·6	+ 18	2·128	+ 8·3	5·3

The method therefore is only of theoretical interest, since it would be too tedious to attempt to resolve mandelic acid by this means.

By using an asymmetric alkyl group Dakin thought he might obtain a more marked difference in the relative rates of hydrolysis of the two forms. To test this point he prepared l-menthyl mandelate, and d-bornyl mandelate. Lipase, however, was without action on these compounds.

Later (J. Physiol. 1905 32 199) Dakin submitted the acetate of phenyl-ethyl-carbinol to partial hydrolysis by lipase, and on examining the products he found them to be

laevo rotatory. In this case the asymmetric carbon atom is present in the alkyl group instead of in the acid part of the molecule. When two grams of ester was hydrolysed to the the extent of 25%, the mixed products of the reaction gave a rotation of - ·31°, so that the difference in the rates of hydrolysis of the two forms was again too small for resolution purposes.

Abderhalden, Sickel and Ueda (Fermentforsch 1923 7 91, J.C.S. Abs. 1923 i 1146) have hydrolysed r-tyrosine ethyl ester with pancreatic lipase. They found preferential hydrolysis of the l-tyrosine ethyl ester, the l-tyrosine being liberated. The d-tyrosine ethyl ester was extracted from the mother liquor.

Emulsin

Fischer (Zeit. Physiol. Chem. 1919 107 184) has obtained the following interesting results with emulsin.

d-mandelonitrile d-glucoside

- after 24 hours was 90% hydrolysed.

l-mandelonitrile d-glucoside

- after 22 hours was 86% hydrolysed.

d-mandelamide d-glucoside

- after 48 hours - no hydrolysis.

l-mandelamide d-glucoside

- after 20 hours - complete hydrolysis.

The action of emulsin on the d-glucosides of -
(1) d- and l- borneol (J.C.S. 1925 127 208), and
(2) d- and l- methyl-n-hexyl carbinol (not yet published)
have now been examined.

In both cases there is a considerable difference in the rates of hydrolysis of the two forms.

Borneol was selected for the first experiment because it is one of the few alcohols which can be obtained in the two optically active forms without carrying through a resolution. Both dextro and laevo borneol occur naturally, but the latter is the more abundant and can be procured from dealers in essential oils. Attempts to purchase the pure dextro variety proved unsuccessful. It can be prepared, however, from ordinary camphor which is dextro rotatory. On reduction, camphor yields a mixture of d-borneol and l-isoborneol. This is the "borneol" of commerce. The dextro form of borneol can be conveniently isolated from this product (see experimental part).

With emulsin both d-bornyl d-glucoside and l-bornyl d-glucoside hydrolyse slowly, and readings were taken over a period of about fifteen hours. In each case there was an interval at the beginning when the reaction did not follow the unimolecular law. This has been found with other en-

zymes as well (c.f. Armstrong, Proc. Roy. Soc. 1904 73 500) Readings are therefore not recorded during the first few hours. The hydrolysis constants obtained had the following values -

d-bornyl d-glucoside	·00010
l-bornyl d-glucoside	·00034

so that the l-bornyl d-glucoside is hydrolysed 3·4 times as rapidly as the d-bornyl d-glucoside.

(2) The work was then extended to the glucosides of d- and l- methyl-n-hexyl carbinol. This alcohol had to be resolved before preparing the glucosides.

In this case, the hydrolysis proceeds much more rapidly and there is a very much greater difference in the relative rates of hydrolysis, than with the glucosides of borneol. The following values were obtained for the reaction constants-

l-methyl-n-hexyl carbinyl d-glucoside	·0055
d-methyl-n-hexyl carbinyl d-glucoside	·0466

so that the compound of the l- alcohol is hydrolysed 8·5 times as rapidly as the corresponding glucoside of the d- alcohol.

It is remarkable that in the case of the bornyl glucosides the form containing the l- alcohol is the more

readily hydrolysed, but with the methyl-n-hexyl carbinyl glucosides it is the compound of the d- alcohol which hydrolyses more rapidly.

With the large difference in the rates of hydrolysis found in (2) above, it should be a practical proposition to use it for the purpose of resolving methyl-n-hexyl carbinol.

It is hoped to extend this line of investigation to other substances, as it may prove useful for resolving some compounds which do not lend themselves readily to the other methods at present available.

It would be a great advantage if the glucosides could first be prepared by the synthetic action of emulsin as used by Bourquolet and Bridel (Ann. Chim. Phys. 1913 [8] 29 145). This synthesis would almost certainly be asymmetric. (A similar type of asymmetric synthesis is described by Rosenthaler, Biochem. Zeit. 1908 14 238. He prepared active benzaldehyde cyanhydrin from a mixture of hydrocyanic acid and benzaldehyde by the action of emulsin.) By a careful combination of synthesis and hydrolysis, without actually isolating the glucoside, it might be possible to bring about resolution. (c.f. the experiment of Marckwald and McKenzie, Ber. 1899 32 2130, in which racemic mandelic acid was heated with l-menthol, but the reaction was stopped after a lapse

of time insufficient to let it go to completion. The l-menthol was found to combine more slowly with the l-mandelic acid than with the d- acid. It was also shown that the compound which was formed more rapidly was also the more rapidly hydrolysed.)

Experimental

HYDROLYSIS OF THE d-GLUCOSIDES OF d- AND l-BORNEOL WITH EMULSIN.

Preparation of the d-Borneol

The d-borneol was obtained from commercial "borneol" which is a mixture of d-borneol and l-isoborneol resulting from the reduction of camphor. The isoborneol is readily dehydrated by zinc chloride, whilst borneol is not attacked. (Pickard and Littlebury, J.C.S. 1907 91 1977)

" 100 gms. of the commercial product are dissolved in 80 gms. of benzene, and boiled with 50 gms. of zinc chloride for three hours. The solution is then washed with acidified water,and fractionally distilled."

The d-borneol thus obtained was crystalised from petroleum ether and had $[\alpha]^{20}_{5461}$ = + 42·14, calculated from a 15·4% solution in alcohol.

The l-borneol was purchased from Schimmel & Co., Leipzig, and had $[\alpha]^{20}_{5461}$ = - 42·20, calculated as in the case of the d-compound from a 15·4% solution in alcohol.

Preparation of the Glucosides

The β-glucosides were prepared by the method of Koenigs and Knorr (Ber. 1901 34 957) in which β-tetra acetylbromo-

glucose (acetobrom glucose) in ethereal solution is treated with an excess of the alcohol in presence of freshly precipitated silver carbonate. The acetyl groups are subsequently removed by means of barium hydroxide.

The acetobrom glucose is conveniently prepared from β-glucose pentacetate by the action of hydrobromic acid in acetic acid solution. (Fischer, Ber. 1916 49 584) .

The d-bornyl d-glucoside has been prepared by Fischer and Raske (Ber. 1909 42 1473), and the l-bornyl d-glucoside has also been obtained. (Hämäläinen, Biochem. Zeit. 1913 50 217).

Emulsin (2·5 g.) was mixed with 180 c.c. of water, and was allowed to stand for some time in a thermostat at 37°. The solution was filtered before use.

Hydrolysis Experiment

A small quantity (0·3 g.) of each glucoside (which contains one molecule of water of crystallisation) was placed in a 50 c.c. flask in a thermostat at 37°. The hydrolysis of the d-bornyl d-glucoside was started by filling the flask to the mark with the emulsin solution, and an hour later the hydrolysis of the l-bornyl d-glucoside was commenced. Two c.c. were removed at intervals, and the amount of glucose present was determined by MacLean's method for estimating

the sugar in blood (Biochem. J. 1919 13 135). In this method, the solution containing the glucose is boiled with an alkaline copper solution in which potassium iodide and iodate are present. Careful regulation of the heating is necessary, and this is accomplished by inserting a manometer between the gas mains and the burner. The solution in which the cuprous oxide is suspended is cooled, treated with a slight excess of hydrochloric acid, and the free iodine is titrated with sodium thiosulphate.

The hydrolysis constants are calculated from the usual formula for unimolecular reactions.

$$K = \frac{2{\cdot}30}{t_y - t_x} \log \frac{c_x}{c_y}$$

d-Bornyl d-glucoside.

Time in Mins	Glucose mg. in 2 c.c.	Glucoside mg. in 2 c.c.	$K \times 10^5$
270	0·70	10·13	
			10
330	0·77	10·01	
			9
515	0·82	9·92	
			11
613	0·89	9·80	
			10
860 (=t_y)	1·03	9·55	Average = 10

l-Bornyl d-glucoside

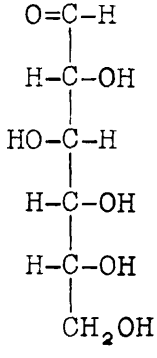
Time in Mins	Glucose mg. in 2 c.c.	Glucoside mg. in 2 c.c.	$K \times 10^5$
180	0·87	9·83	
			34
303	1·12	9·39	
			33
361	1·22	9·22	
			33
435	1·33	9·03	
			35
673 (=t)	1·74	8·31	Average = 34

Hence emulsin hydrolyses l-bornyl d-glucoside 3·4 times as rapidly as d-bornyl d-glucoside.

HYDROLYSIS OF THE d- GLUCOSIDES OF d- AND METHYL-n-HEXYL CARBINOL WITH EMULSIN.

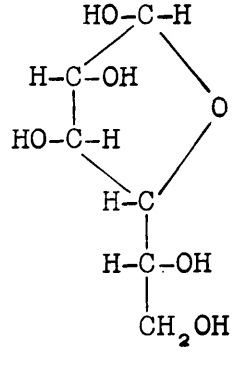
Preparation of the Glucosides

The glucosides of methyl-n-hexyl carbinol have not previously been prepared, so a quantity of the alcohol as obtained commercially was first used to test the method before actually resolving the alcohol into its optical isomers. The commercial alcohol is not absolutely inactive, it usually has a very small rotation. The sample employed in these experiments had $\alpha^{20}_{5461} = -\cdot 21$ for a decimetre tube. The following is an outline of the method.



Glucose

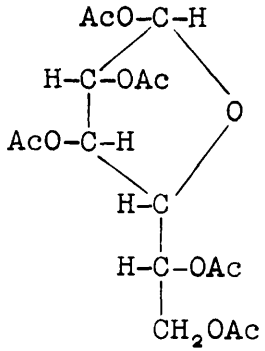
or writing it in the now more usual Butylene oxide formula



β-Glucose

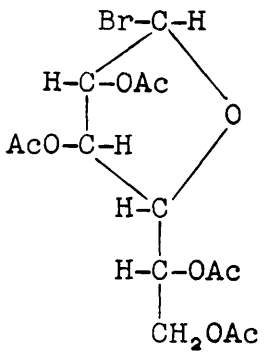
on acetylation

(Ac = C_2H_3O)



β-Glucose pentacetate

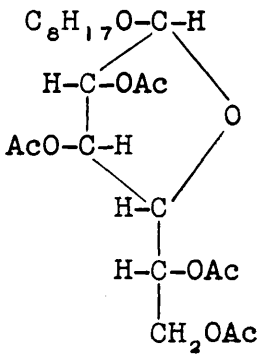
H Br



Acetobrom-glucose

$C_8H_{17}OH$

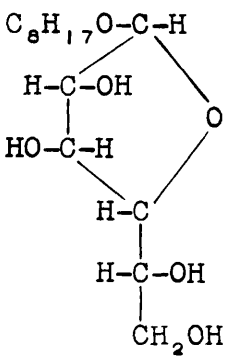
in presence of silver carbonate



β-Tetracetyl methyl-n-hexyl carbinyl d-glucoside

$Ba(OH)_2$

removes acetyl groups



β-Methyl n-hexyl carbinyl d-glucoside

β-Tetracetyl methyl-n-hexyl carbinyl d-glucoside

30 g. of the alcohol were dissolved in 100 c.c. of anhydrous ether, and 5 g. of acetobrom glucose together with 5 g. of freshly prepared and thoroughly dried silver carbonate

were added. The mixture was shaken for three days. A further 5 g. of acetobrom glucose and 5 g. of silver carbonate were then put in, and the shaking was continued for another three days. Two more lots of acetobrom glucose and silver carbonate were added at intervals of three days, so that in all there was 20 g. of acetobrom glucose and 20 g. of silver carbonate added during an interval of twelve days. The ether was then evaporated off, and the uncombined alcohol was removed by steam distillation. The tetracetyl compound was crystallised first from aqueous alcohol and finally from alcohol. It separated from the latter solvent in long needles. M.P. = 95°.

The substance was easily soluble in ethyl alcohol, acetone, ether and benzene; moderately soluble in petroleum ether; and practically insoluble in water. It gave the following results on analysis.

Weight of substance taken = ·1810 g.

Weight of CO_2 found = ·3794 g.

Weight of H_2O found = ·1251 g.

% C = 57·2

% H = 7·7

$C_{22}H_{36}O_{10}$ requires % C = 57·4 and % H = 7·8.

β-Methyl n-hexyl carbinyl d-glucoside

4 g. of β-tetracetyl methyl-n-hexyl carbinyl d-glucoside was put into a bottle containing 240 c.c. of water, 75 c.c. of ethyl alcohol, and 16 g. of barium hydroxide. The mixture was kept at 55-60° for five hours. It was shaken frequently until the whole passed into solution. The barium hydroxide remaining was then precipitated by bubbling in carbon dioxide. The barium carbonate was filtered off, and the solution was evaporated to dryness. The residue was extracted with alcohol, and the solution again taken to dryness. The glucoside was dissolved in anhydrous ether, and was allowed to crystallise.

The substance was found to be easily soluble in water, alcohol and acetone; moderately soluble in ether; not very soluble in benzene; and insoluble in petroleum ether. On analysis the following results were obtained.

Weight of substance taken = ·1842 g.

Weight of CO_2 found = ·3640 g.

Weight of H_2O found = ·1556 g.

% C = 53·9

% H = 9·4

$C_{14}H_{28}O_6 \cdot H_2O$ requires % C = 54·2 and % H = 9·2.

Resolution of the methyl-n-hexyl carbinol

A quantity of the methyl-n-hexyl carbinol was resolved by the method described by Pickard and Kenyon (J.C.S. 1907 91 2058),and modified by Kenyon (J.C.S. 1922 121 2540).

Methyl-n-hexyl carbinyl hydrogen phthalate was first prepared and dissolved in acetone. Brucine was added, and the mixture was warmed until solution was complete. On cooling, the d-methyl-n-hexyl carbinyl l-brucine phthalate separated out first. The brucine was removed, and the two esters were each crystallised from acetic acid. Finally the esters were hydrolysed with sodium hydrate solution, and the optically active alcohols were steam distilled off, dried and distilled.

For the d-alcohol α^{18}_{5461} = + 8·26 (in a 1 d.m. tube)

For the l-alcohol α^{18}_{5461} = - 8·78 (in a 1 d.m. tube)

The d-glucosides of the d- and l- forms of the alcohol were then prepared as described above for the racemic alcohol.

Hydrolysis Experiment

A small quantity (about 0·1 g.) of each glucoside was weighed into a 25 c.c. flask which was placed in a thermostat

at 37°. The enzyme solution was made up by mixing 0·5 g. of emulsin with 60 c.c. of water, filtering and allowing to come to 37° in the thermostat.

The hydrolysis of the d-methyl-n-hexyl carbinyl d-glucoside was commenced by filling the graduated flask to the mark with the emulsin solution. After a few readings had been taken, the hydrolysis of the l-methyl-n-hexyl carbinyl d-glucoside was started. MacLean's method was again employed for estimating the amount of glucose set free.

The constants were calculated from the unimolecular reaction formula (see page 19)

d-Methyl-n-hexyl carbinyl d-glucoside

"A" = number of c.c. of N/100 $Na_2S_2O_3$ equivalent to the amount of glucose set free in 1 c.c. of solution.

"c" = number proportional to concentaation of unchanged glucoside present at time shown.

"K" = the hydrolysis constant.

Time in mins	A	c	K
0	0	7·85	
18	4·12	3·73	·0468
33	6·00	1·85	·0467
48	6·93	·92	·0470
63	7·40	·45	·0464
78	7·55	·30	·0459
91·5	7·72	·13	Average ·0466
∞	7·85		

1-Methyl-n-hexyl carbinyl d-glucoside

Time in mins	A	c	K
0	0	7·93	
105	3·20	4·73	·0055
150	4·24	3·69	·0056
180	4·75	3·17	·0055
278	6·10	1·85	·0056
311	6·43	1·50	·0055
405	7·02	·91	Average ·0055
∞	7·93		

Hence d-methyl-n-hexyl carbinyl d-glucoside is hydrolysed 8·5 times as rapidly as l-methyl-n-hexyl carbinyl d-glucoside.

THE OXIDATION OF CEDRENE WITH HYDROGEN PEROXIDE.

THE OXIDATION OF CEDRENE WITH HYDROGEN PEROXIDE.

Historical

The products of the cedar tree (Juniperus virginiana) have proved of service to mankind from very early times. The oil was used by the Egyptians for embalming purposes, and the wood was employed in the construction of Solomon's temple. More recently the oil has been found suitable for microscopic immersions, and pencils are now extensively manufactured from the wood. Our knowledge of the chemistry of the oil is, however, still very incomplete, and little has been established regarding the constitution of its constituents.

Walter (Ann. Phys. Chim. [3] 1841 1 498, 1843 8 354) fractionally distilled the oil and isolated two products.

(1) A solid camphor like substance.

(2) A hydrocarbon.

On treating (1) with phosphorus pentoxide, he obtained a hydrocarbon which gave the same analysis as (2), and which he called cedrene. Later the name was used as a general term for hydrocarbons of the formula $C_{15}H_{24}$, but these are now designated as sesquiterpenes, and "cedrene" is reserved for the $C_{15}H_{24}$fraction which occurs naturally in cedarwood oil.

Gladstone (J.C.S. 1891 59 290) showed from refractivity results that cedrene contains one double bond. The molecular refraction (calculated from the formula $\frac{n_\lambda - 1}{d} \cdot M$) gave the value 108·52, as compared with 108·4 obtained by the addition of atomic refractivities plus the exaltation due to one double bond. This was confirmed later by the Lorenz and Lorentz formula.

Chapman and Burgess (Proc. Chem. Soc. 1896 12 140) found that cedrene (d_{15}^{15} = ·9359, α_D = 60° in a 1 d.m. tube μ_D = 1·5015) combined readily with hydrochloric acid and bromine, but they were unable to isolate any definite addition products. They obtained negative results also with nitrosyl chloride and the oxides of nitrogen.

Rousset (Bull. Soc. Chim. 1897 17 485) showed that the solid constituent of cedarwood oil was a tertiary alcohol which he called cedrol.

On oxidising cedrene with chromic acid in acetic acid solution, he obtained a ketone $C_{15}H_{24}O$ (cedrone). On reduction this gave an alcohol $C_{15}H_{26}O$ to which the name iso-cedrol was given.

By acting on cedrene with sulphuric acid in acetic acid solution, he thought that he might be able to add on the elements of water, but this hydration experiment proved unsuccessful

Semmler and Hoffmann (Ber. 1907 40 3519) repeated the work of Rousset. They found that cedrone has the formula $C_{15}H_{22}O$ and not $C_{15}H_{24}O$ as stated by Rousset. The alcohol $C_{15}H_{26}O$ produced by reduction was, however, the same as that previously obtained, but they called it dihydro-iso-cedrol.

They also studied the action of potassium permanganate on cedrene. Three products were isolated.

(1) Cedrene ketonic acid $C_{15}H_{24}O_3$
B.P. 215-222° / 11m.m. from which they prepared a semi-carbazone, oxime and methyl ester.

(2) Cedrene keto-aldehyde or diketone $C_{15}H_{24}O_2$
B.P. 165° / 10 m m. which gave a disemicarbazone.

(3) Cedrene glycol $C_{15}H_{26}O_2$
A solid M.P. 160°.

Semmler and Risse (Ber. 1912 45 355) found that Ozone gave the same products (1) and (2) as permanganate, but not the glycol. In addition a ketone $C_{14}H_{24}O$ or $C_{14}H_{22}O$ was produced. Oxidation of the keto-acid with NaOBr gave a dicarboxylic acid $C_{15}H_{22}O_4$ M.P. 182·5°.

Semmler and Mayer (Ber. 1912 45 786 and 1384) discovered two additional sesquiterpene alcohols in cedarwood oil.

The first occurred to the extent of 3%, and they found it to be a primary alcohol and called it cedrenol. It had the following constants.

B.P. 166-169° / 9·5 m.m., d^{20} = 1·0083, α_D^{20} = 0, and n_D^{20} = 1·5212.

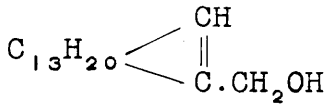
The second was a liquid saturated alcohol, a physical isomer of cedrol, which they called pseudo-cedrol.

B.P. 147-152°, d^{20} = ·9964, α_D^{20} = 21·5° (in a 1 d.m. tube)

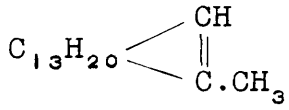
Cedrenol gave the following results

$$C_{15}H_{23}OH \xrightarrow{P\,Cl_5} C_{15}H_{23}Cl \xrightarrow{Na} C_{15}H_{24}$$

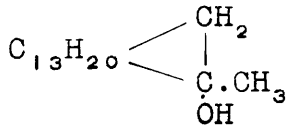
Also cedrol and pseudo-cedrol on dehydration with formic acid each gave a hydrocarbon $C_{15}H_{24}$. Oxidation of each of these three hydrocarboms with ozone in acetic acid produced a keto-acid. On subsequent oxidation with NaOBr all three keto-acids gave the same dicarboxylic acid $C_{15}H_{22}O_4$ M.P. 182·5. This is the same melting point as that of the dicarboxylic acid obtained from natural cedrene. The authors conclude, therefore, that the "cedrene" from these four sources is essentially the same substance. They suggest that the relationship of cedrenol and cedrol to cedrene may be represented by the formulae.



Cedrenol

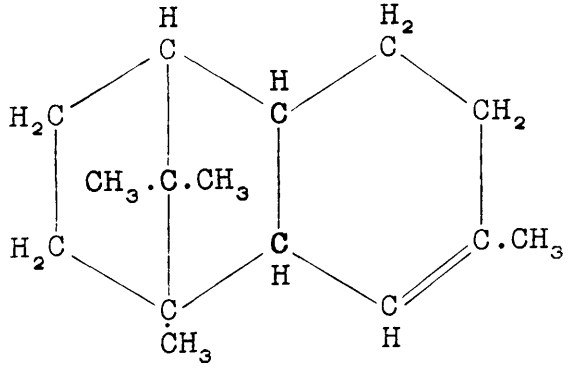


Cedrene



Cedrol

The tricyclic nature of cedrene is shown by the fact that it forms a dihydride $C_{15}H_{26}$ on catalytic hydrogenation, and the following structural formula has been put forward by Semmler and Stenzil (Ber. 1914 46 3).



Cedrene

At the suggestion of Professor G. G. Henderson, the author examined the action of hydrogen peroxide on cedrene. Previously this reagent had been extensively employed in the Terpene series. See for example

Henderson and Sutherland J.C.S. 1911 99 1539 (Camphene)
" " " J.C.S. 1912 101 2288 (α-pinene)
" " Caw J.C.S. 1913 103 1543 (Bornylene)
" " Robertson J.C.S. 1923 123 1849 (Sabinene)
" " Chisholm J.C.S. 1924 125 107 (β-pinene)

Oxidation of Cedrene with Hydrogen Peroxide

As a result of the present investigation two products were isolated.

(1) A solid M.P.165°, which proved to be cedrene glycol. This had already been obtained by Semmler and Hoffmann as the result of potassium permanganate oxidation.

(2) A liquid product B.P. 154-156° / 12 m.m.

$d_4^{20} = 1{\cdot}0007$, $n_D^{20} = 1{\cdot}5009$, $[\alpha]_{5461}^{20} = -78$

Although attempts to esterify it proved unsuccessful, it reacted with sodium to give a solid derivative. When treated with methyl iodide this gave an oil B.P. 140-145° / 10 m.m. which may be the methyl ether. The product was therefore thought to be a tertiary alcohol.

Its physical constants do not agree with those of any of the natural alcohols already described. It is also of interest as being the first alcohol which has been prepared from cedrene. Confirmation of its alcoholic nature is, however, desirable.

EXPERIMENTAL

Preparation of Pure Cedrene

The commercial "cedrene" was first distilled over sodium under reduced pressure. The resulting distillate was then fractionated, the portion boiling at 123-124° at 9 m.m. was taken as pure. It had the following physical properties.

$$d_4^{20} = \cdot 9351$$

$$[\alpha]_{5461}^{20} = -70 \cdot 04$$

$$n_D^{20} = 1 \cdot 50167$$

Oxidation of Cedrene with Hydrogen Peroxide

50 g. of cedrene were dissolved in glacial acetic acid (about 300 c.c.) and 60 g. of 30% hydrogen peroxide were added. The addition of the peroxide caused a separation into two layers, but on warming, the top layer went very gradually into solution. The mixture was kept at 40-50° for three days, i.e. until a homogeneous solution had been obtained. This solution was diluted with water, and sodium carbonate was added in small quantities until the acetic acid was almost neutralised. The yellow oil which separated

was extracted with ether. After washing and drying, the ether was distilled off, and the oily residue was warmed with aqueous sodium carbonate. The neutral products were removed with ether . The remainder was acidified, and also extracted with ether, but no acid product was found to be present.

Neutral Products

Portions were tested for aldehydes and ketones with sodium bisulphite and semicarbazide hydrochloride, but neither was found to be present.

The neutral products were then hydrolysed with 20% methyl alcoholic potash for half an hour. The excess of alkali was converted to carbonate by passing in carbon dioxide. The methyl alcohol was then distilled off, a quantity of water was added to keep the solids in solution, and the whole was extracted with ether. The remaining aqueous solution was acidified and also extracted with ether. A small quantity of a brown liquid - mostly acetic acid was obtained. The ether was then removed from the remainder of the product. A quantity was steam distilled, but it was found that it all passed over with the steam, and so the method could not be used for separation purposes. Fractional distillation under reduced pressure had therefore to be

resorted to. The following fractions were collected at 12 m.m.

(1) Under 150°

(2) 150 - 160°

(3) 160 - 180°

(4) Over 180°

(3) on redistillation gave more of (2) and (4). (2) was fractionated and gave an almost colourless liquid, which boiled at 154 - 156° at 12 m.m.

Properties of Liquid Product

B.P. / 12 m.m. = 154 - 156°

d_4^{20} = 1·0007

$[\alpha]^{17}_{5461}$ = - 78·0

n_D^{20} = 1·5009

On analysis the following results were obtained.

	(1)	(2)
Weight of substance taken	= ·1874 g.	= ·1878 g.
Weight of CO_2 found	= ·5631 g.	= ·5638 g.
Weight of H_2O found	= ·1925 g.	= ·1895 g.
	% C = 82·0	% C = 81·9
	% H = 11·4	% H = 11·2

$C_{15}H_{26}O$ requires % C = 81·1

% H = 11·7

and $C_{15}H_{24}O$ requires % C = 81·8

% H = 10·9

The liquid was saturated

It reacted with sodium in dry benzene solution on warming. A solid was deposited on standing. When treated with methyl iodide this gave an oil B.P. 140 - 145° / 10 m.m. which is probably the methyl ether.

Attempts to prepare the following derivatives proved unsuccessful.

(1) A p.nitrobenzoate

(2) A phenylurethane

Oxidation with 1% potassium permanganate in aqueous acetone reacted slowly, but no solid derivatives were obtained.

The liquid would therefore on the evidence given above appear to be a tertiary alcohol.

The Solid Product

The fraction of oxidation products boiling over 180° at 12 m.m. was a very viscous yellow oil. It was diluted with acetone and kept in a cool place until crystals were deposited. These were recrystallised from acetone, and gave long needles which melted at 165°.

On analysis the following results were obtained

Weight of substance taken = ·1585 g.

Weight of CO_2 found = ·4370 g.

Weight of H_2O found = ·1572 g.

% C = 75·2

% H = 11·0

$C_{15}H_{26}O_2$ requires % C = 75·6 and % H = 10·9

There seems therefore to be no doubt that this compound is cedrene glycol, and is identical with the product obtained by Semmler and Hoffmann (Ber. 1907 40 3522) when they oxidised cedrene with potassium permanganate.

AN OPTICAL METHOD FOR DETERMINING THE SOLUBILITY OF SPARINGLY SOLUBLE SUBSTANCES.

AN OPTICAL METHOD FOR DETERMINING THE SOLUBILITY OF SPARINGLY SOLUBLE SUBSTANCES.

The methods available for determining the solubility of sparingly soluble substances are of limited application. Electrical measurements are restricted to electrolytes and Chemical analysis is useful for only a few substances. The Optical method now described can, however, be employed more generally. It makes use of the interferometer, and a brief account of the origin of the instrument will first be given.

Young's Experiment

Thomas Young was the first to produce interference bands. His experimental arrangement is described as follows (Phil. Trans. 1804 94 2)

"I made a small hole in a window shutter, and covered it with a piece of thick paper which I perforated with a fine needle. For greater convenience of observation I placed a small looking glass without the window shutter, in such a position as to reflect the sun's light, in a direction nearly horizontal, upon the opposite wall, and to cause the cone of diverging light to pass over a table on which were several little screens of card paper: I brought into the sunbeam a slip of card, about one thirtieth of an inch in breadth, and observed its shadow, either on the wall or on other cards

held at different distances. Besides the fringes of colour on each side of the shadow, the shadow itself was divided by similar parallel fringes of smaller dimensions differing in number, according to the distance at which the shadow was observed, but leaving the middle of the shadow always white. Now these fringes were the joint effects of the portions of light passing on each side of the slip of card, and inflected, or rather diffracted into the shadow. For a little screen being placed either before the card or a few inches behind it, so as either to throw the edge of the shadow on the margin of the card or to receive on its own margin the extremity of the shadow of the card, all the fringes which had before been observed in the shadow on the wall immediately disappeared."

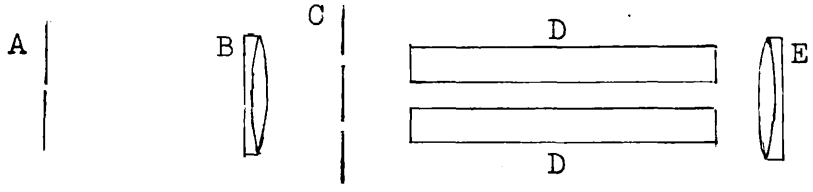
Young's work was most unmercifully attacked in the then highly popular Edinburgh Review by Brougham (who afterwards became Lord Chancellor) in three articles celebrated for their stinging satire. Unfortunately, these appear to have diverted the attention of British men of Science from Young's views on interference, and it is in France that we find the next development of the wave theory of light. Byron makes an interesting reference to the critic in his " English Bards and Scottish Reviewers."

"Yet mark one caution, ere thy next Review
Spread its light wings of Saffron and of Blue
Beware lest blundering Brougham spoil the sale,
Turn Beef to Bannocks, Cauliflowers to Kail."

Arago's Interferometer

In 1816 Arago repeated Young's experiment (Oeuvres Completes 1858 Vol 10 312). He found that instead of the little screen a piece of glass also caused the interference bands to disappear, when placed in the path of the light passing on one side of the slip of card. This led him to try very thin pieces of glass produced by heating a bulb, and then blowing it out. The bands did not then disappear, they were simply displaced. The displacement varied with the thickness of the glass. He then tried other transparent substances, and found that the displacement also depended on the refractive index. This discovery led him to improve Young's apparatus, and to make use of the displacement of the interference bands for measuring small differences in refractive index. His arrangement is shown on next page.

Using tubes one metre long with (1) both tubes filled with dry air, and (2) dry air in one tube and moist air in the other, there was a displacement of 1·25 bands.



A Very narrow slit
B Collimating object glass
C Double slit
D Two tubes of same length
E Achromatic telescope
F Cylindrical lens

Rayleigh (Collected Works Vol. 4, 364) used this type of interferometer for his work on the refractive indices of gases, and it is now usually known as the "Rayleigh Interferometer". The instrument suffers from two disadvantages.

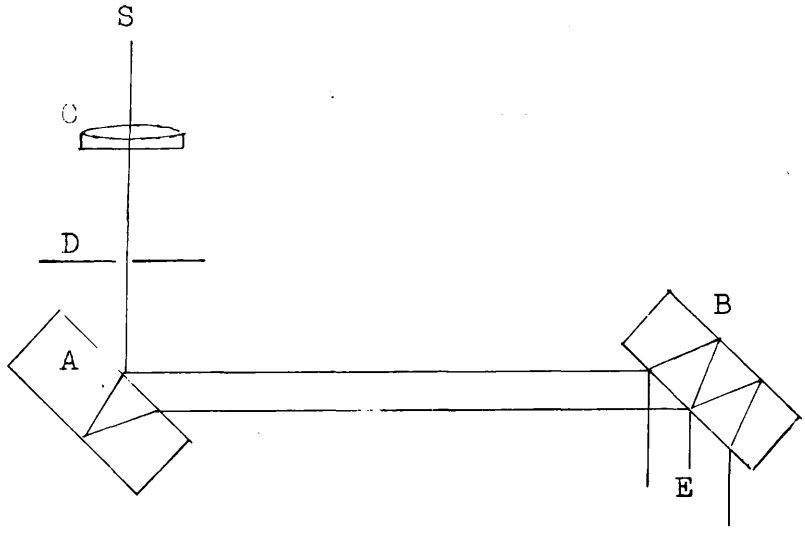
(1) An extremely narrow slit has to be used, and unless a very bright source of light is available the illumination is poor.

(2) The bands are close together even under the best conditions. Their distance apart is controlled by the distance between the slits C, being inversely proportional to this length.

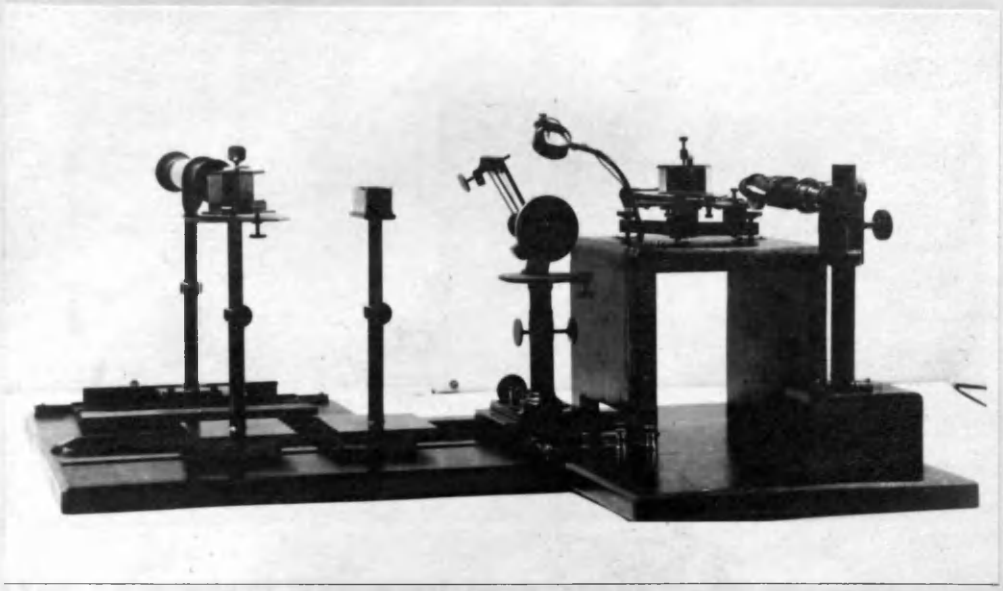
The Jamin Interferometer

Jamin (Ann. Chim. Phys. 1858 [3] 52 163) constructed an interferometer which overcame these difficulties. This is the type of instrument which the author constructed, and has

used for determining the solubilities of some sparingly soluble substances. It may therefore be briefly described.



A and B are two rectangular blocks of exactly the same thickness. They are cut from the same piece of optical glass, and the back surface of each is silvered. Light from the source S is rendered parallel by the collimating object glassC, and after passing through a small circular hole in the diaphram D arrives at the surface of the first block where part is reflected and part refracted. The remaining paths of the two beams are shown in the diagram. By careful rotation of B, so as to render the two blocks very nearly parallel, interference bands appear at E, and are conveniently observed by means of a reading telescope.



The early investigators used white light as source of illumination. At first sight it would appear to be very convenient because it gives a white central band which can be taken as zero. Later work has shown, however, that this white band undergoes a gradual shift owing to differences in the dispersion of the contents of the two tubes, and a correct-ion has to be introduced. It is therefore simpler to work with monochromatic light. We have then the relation

$$L(\mu_1 - \mu_2) = s.\lambda$$

where

L = Length of tubes
μ_1 = refractive index of contents of first tube
μ_2 = refractive index of contents of second tube
s = number of bands displaced
λ = wave length of light used.

Procedure for a Gas

The two tubes are first evacuated, and the telescope cross wire is placed on a particular band. When a gas is allowed to enter one of the tubes, there is a gradual displacement of the bands, and the number which pass is noted.

Jamin Compensator

Instead of counting the bands, the same band can be kept on the cross wire all the time by means of a compensator. Jamin's arrangement consists of two pieces of optical glass fixed at a small angle to each other, and placed so that one plate is in each beam. The plates rotate on a horizontal axis which is supplied with a scale (see photograph on previous page).

EXPERIMENTAL

A Jamin interferometer, as previously described, was used with Mercury green light (λ = 5461) as source of illumination. A special form of double cell was required, however, in which liquids could be circulated through each compartment. It was cut from a rectangular block of brass (2·5 cm. broad) and was fitted with inlet and outlet tubes of the same metal. The sides were made of the best quality of optical glass cemented in position. Both compartments of the cell were first filled with water, and the compensator was adjusted so that the cross wire of the observing telescope coincided with one of the interference bands. When a dilute aqueous solution was gradually allowed to displace the water in one half of the cell, there was a movement of the bands. By means of a fine adjustment on the compensator, the same band could be kept on the telescope cross wire.

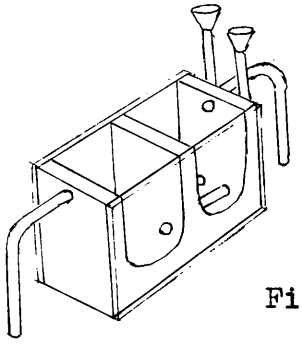


Fig 1

Over the range considered, the compensator readings were found to be proportional to the number of bands displaced, and therefore to the difference in refractive index between

the contents of the two compartments of the cell (Table 1 and Fig. 2)

As a preliminary it was necessary to investigate the nature of the curves connecting compensator readings with concentration. To do this, a number of saturated solutions of substances slightly soluble in water were prepared at room temperature. A series of dilutions of each was made, and these together with a quantity of water were allowed to stand till all were at the same temperature. Both divisions of the cell were then filled with water, and the zero reading was taken. Water was allowed to circulate through one compartment, and the most dilute solution through the other. When no further movement of the bands took place, the reading was noted. The next solution was then allowed to displace the more dilute, and so on till the saturated solution was reached. A number of results are shown in Table 2 and Fig. 3. All substances so far investigated give straight lines with the exception of benzene. In view of this exception it will always be necessary to plot a graph of percentage saturation against compensator readings as a preliminary to a solubility determination by this method. When the graph is a straight line, only one weighing is necessary in order to calibrate the concentration scale,and if the reading for

the saturated solution is determined, the weight of the solute present in it can be found graphically by extrapolation. In practice it is advisable to prepare one or more dilutions from the solution of known concentration so that there may be a gradual movement of the bands without blurring. Should the graph not be a straight line, a number of weighings would be necessary and the method would lose its merit of simplicity.

When a solubility is required at a temperature other than room temperature, a saturated solution is prepared at that temperature, separated off and if necessary a known quantity of water is added so that the solute will remain completely in solution at the temperature of the room in which the readings are taken. The saturated solutions were prepared in stoppered bottles which were rotated in a thermostat with excess of solute for several hours at the required temperature. Table 3 shows a number of results.

Water has been used as solvent in these experiments, but the method could be extended to other solvents if not too volatile.

The author desires to thank Miss Janet M. Henderson for assistance with part of the experimental work.

Table 1

Compensator Readings	Bands
10	0
13·6	2
17·0	4
20·5	6
24·0	8
27·7	10
31·5	12
35·0	14
39·0	16
43·0	18
46·5	20

Graph showing the relation between compensator readings and the number of bands displaced

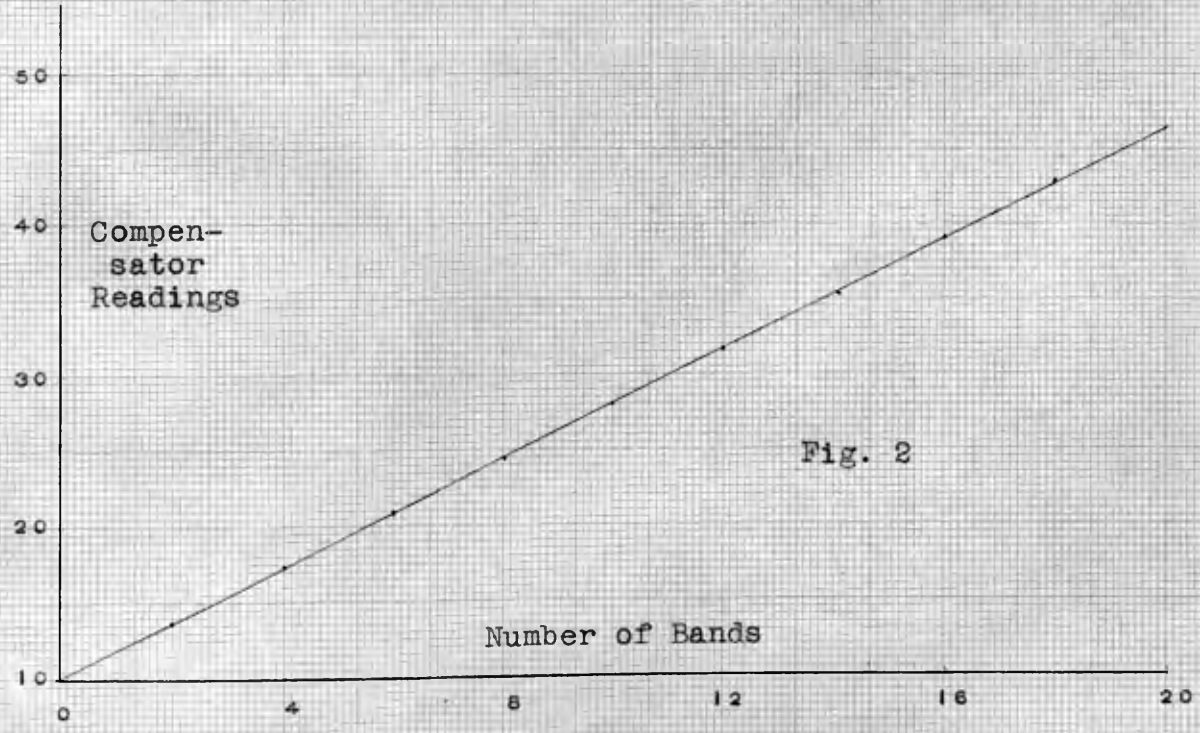


Fig. 2

Table 2.

Substance	Compensator Readings for % Saturation										
	0%	10%	20%	30%	40%	50%	60%	70%	80%	90%	100%
Benzene	10·0	12·0	14·3	16·2	18·5	20·8	23·5	26·5	32·0	38·8	45·0
Camphor	10·0	12·8	16·3	19·8	23·6	26·7	29·0	32·2	35·6	38·7	43·2
Sec. Octyl Alcohol	10·0	12·4	14·0	15·5	16·8	18·8	20·0	21·5	22·4	24·5	26·0
Borneol	10·0	11·5	12·8	14·1	15·3	16·7	18·3	19·4	20·8	22·3	24·0
Methyl Salicylate	10·0	11·0	12·2	13·5	15·0	16·4	17·9	19·1	20·3	21·6	23·0
Naphthalene	10·0	11·0	11·5	12·0	12·6	13·7	14·3	14·8	15·5	16·8	18·1

Fig. 3

Graph showing the relation between compensator readings and % saturation.

A Benzene
B Camphor
C Sec. Octyl Alcohol
D Borneol
E Methyl Salicylate
F Naphthalene

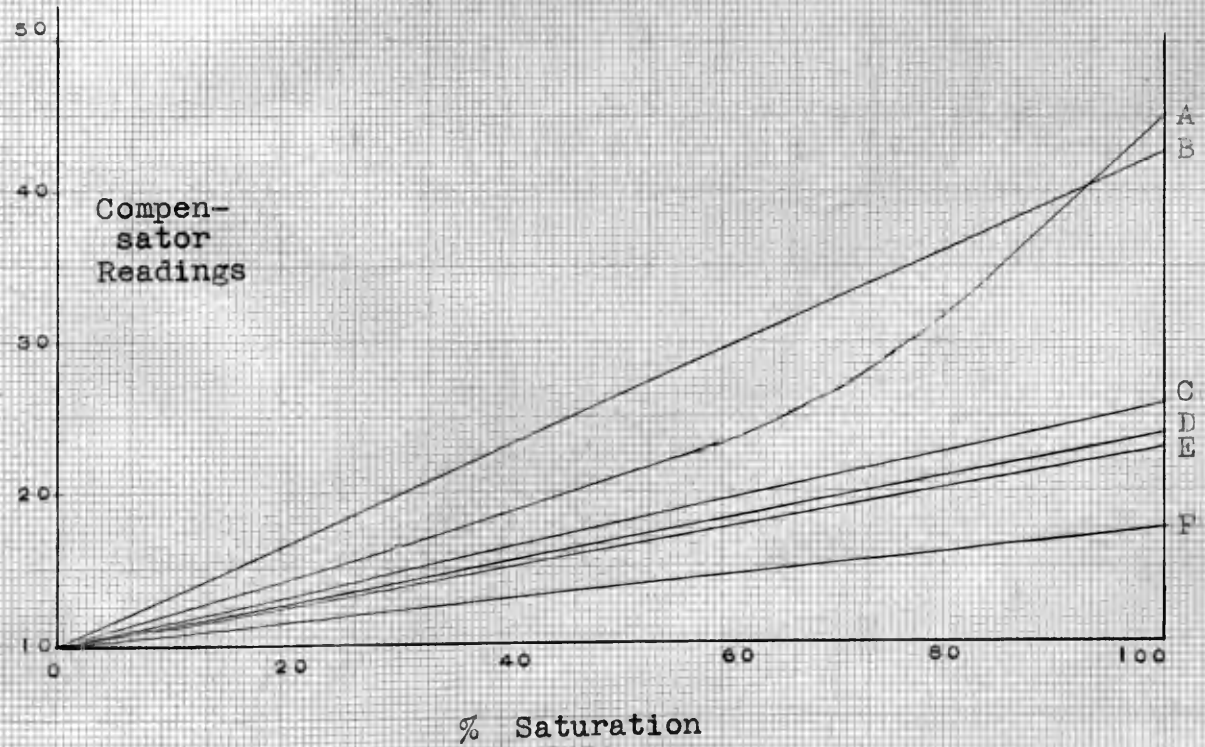


Table 3

Substance	Gms. substance dissolved in 1000 cc of water.	Compensator Readings.				Temp °C	Solubility Gms. per 1000 cc of water.
		Soln A	A diluted 50 %	Sat. soln B	B diluted 50 %		
Naphthalene	·0200	14·0	12·0	14·5		15	·022
					14·0	25	·040
Lead sulphate	·0250	11·4	10·7	11·8		15	·032
Sec.octyl alcohol	1·0407	19·0	14·5	23·5		15	1·508
				21·5		25	1·280
Borneol	·5000	18·3	14·3	21·4		15	·693
					16·2	25	·740