CXL.—*The Resolution of β -Hydroxybutyric Acid into its Optically Active Components.*

By ALEX. MCKENZIE.

OF the three β -hydroxybutyric acids, two have previously been described. The inactive acid was prepared by J. Wislicenus (*Annalen*, 1869, 149, 205) by the reduction of ethyl acetoacetate with sodium amalgam. The *l*-acid is an important biological product discovered by Minkowski (*Arch. exp. Path. Pharm.*, 1884, 13, 35, 147) and Külz (*Zeit. Biol.*, 1884, 20, 165) in the urine of patients suffering from *Diabetes mellitus*. It is excreted in very large amount, more particularly when the patient is in the coma stage; Magnus-Levy, for instance, who has lately made an exhaustive study of the bearing of this acid on diabetes, cites one case where he found so much as 119 grams in one day. It is not yet known by what process such large quantities are produced in the organism, although it now appears to be established that the acid is not a product of albuminoid decomposition.

In this paper, the preparation of the *d*- and *l*-acids from the inactive compound is described. The alkaloidal salts formed by the inactive acid are easily soluble in most solvents and are not readily crystallisable. Quinine was found to be the most suitable alkaloid for effecting the resolution, the salt of the *l*-acid being easily crystallisable and less soluble in water than that of the *d*-acid.

l-Acid.

An aqueous solution of inactive β -hydroxybutyric acid (prepared by Wislicenus' method) was neutralised by heating with 180 grams of quinine, and the clear, syrupy solution (450 c.c.) cooled and sown with a nucleus of the *l*-acid-*l*-quinine salt prepared from the acid obtained from diabetic urine. After remaining for 24 hours at 7°, the semi-solid mass was separated, and the filtrate, which in the course of three months did not crystallise, was laid aside to be subsequently treated for the isola-

tion of the *d*-acid. The solid was fractionally crystallised from water. The solutions, when cool, were sown each time with a nucleus of the pure salt and the crystallisations conducted at temperatures varying from 6° to 8°. As the quinine salts of the *d*- and *l*-acids melt within 2° of one another, melting point determinations were of little service as an indication of the progress of the resolution. Further, the difference in optical activity between the two salts is so slight that determinations of $[\alpha]_D$ in successive crops are also of little value. In the special case of this acid, however, the rotation of the naturally-occurring *l*-isomeride has been accurately determined by Magnus-Levy (*Arch. exp. Path. Pharm.*, 1901, 45, 389), so that, when the separation had reached a certain point, portions of successive crops were decomposed in the manner described below and the rotation of the resulting acid examined.

Sixty grams of the *l*-acid-*l*-quinine salt were finally obtained. The water of crystallisation was estimated in the air-dried product :

1·1808, at 100°, lost 0·1876 H₂O. H₂O = 15·8.

C₂₀H₂₄O₂N₂·C₄H₈O₃· $\frac{1}{2}$ H₂O requires H₂O = 15·9 per cent.

The air-dried salt is much more soluble in hot water than in cold, from which it crystallises in needles grouped in rosettes. It is easily soluble in cold ethyl alcohol and in hot ethyl acetate, moderately so in boiling chloroform, and sparingly so in boiling acetone, ether, benzene, or carbon tetrachloride. The air-dried salt melts indistinctly between 60° and 70°; when deprived of its water of crystallisation, it melts at 124·5–125·5°. The specific rotation of the air-dried salt was determined in ethyl alcohol :

$$l = 4, c = 2\cdot814, \alpha_D^{15^\circ} - 14\cdot62^\circ, [\alpha]_D^{15^\circ} - 129\cdot9^\circ.$$

The *l*-acid was prepared from diabetic urine according to Magnus-Levy's method (*loc. cit.*) and was converted into quinine salt. The latter resembled the above described product in crystalline form, melting point, and rotatory power, the specific rotatory power of the air-dried product being $[\alpha]_D^{15^\circ} - 129\cdot0^\circ$ ($c = 2\cdot8052$).

The 60 grams of the quinine salt were dissolved in cold water, decomposed by a slight excess of normal caustic potash solution, and kept at 0° overnight. After removal of the quinine, the filtrate was concentrated and then shaken several times with chloroform to ensure the removal of quinine. After addition of normal sulphuric acid solution equal in amount to the alkali previously added, the β -hydroxybutyric acid was extracted with ether in a continuous extraction apparatus. The acid was obtained first of all as a syrup; an aqueous solution, the concentration of which was determined by titration against

1404 MCKENZIE: THE RESOLUTION OF β -HYDROXYBUTYRIC

normal potassium hydroxide solution with phenolphthalein as indicator gave the following result :

$$l = 4, c = 8.3304, \alpha_D^{16} - 8.31^\circ, [\alpha]_D^{16} - 24.9^\circ.$$

On standing for several months over sulphuric acid in a partial vacuum at about 4° , the syrup solidified. The acid is easily soluble in most of the common solvents and melts at about $45.5-48^\circ$. It was found impossible to get the melting point sharper, as the acid is exceedingly hygroscopic. The specific rotation, where the concentration was estimated by direct weighing, was determined with the following results :

Solvent.	l .	c .	t .	α_D .	$[\alpha]_D$.
Water	2	3.264	20°	-1.62°	-24.8°
Ethyl alcohol.....	„	8.2072	18	2.87	17.5
„ „	„	4.994	16	1.75	17.5
„ „ ..	4	1.9976	„	1.38	17.3

The rotation is only slightly affected by the presence of alkaline uranium nitrate (Walden, *Ber.*, 1897, 30, 2889). A solution with $c = 0.328$ gave $\alpha_D^{16} - 0.21^\circ$ ($l = 2$), whereas for a solution of this concentration in the absence of the alkaline uranium nitrate, the rotation is calculated to be $\alpha_D - 0.16^\circ$ ($l = 2$).

The sodium salt having $[\alpha]_D^{16} - 14.5^\circ$ ($c = 8.518$) was analysed :

0.3430, dried at 110° , gave 0.1963 Na_2SO_4 . Na = 18.6.

$\text{C}_4\text{H}_7\text{O}_3\text{Na}$ requires Na = 18.3 per cent.

For the purpose of obtaining a nucleus of the quinine salt of the l -acid, it has been already mentioned that the acid was prepared from urine by Magnus-Levy's method. The optical activity of the acid so prepared agreed with that recorded by him. Dr. G. A. Finlayson and Dr. J. J. R. Macleod kindly provided specimens of the urine of patients in the coma stage of *Diabetes mellitus*. In the preparation of the acid, it is convenient to perform the requisite ether extractions in a continuous extraction apparatus. Magnus-Levy found $[\alpha]_D^{21} - 24.23^\circ$ ($c = 11.0$) for the acid and $[\alpha]_D^{18-20} - 14.15^\circ$ ($c = 7.317$) for the sodium salt, water being the solvent in each case. These results accordingly establish the identity of the naturally-occurring acid with the synthetic product.

The values of the specific rotatory power of the acid as determined by Magnus-Levy and myself are in excess of those by Minkowski and Klz and are very little altered by changes of temperature and con-

centration. The solutions do not exhibit the varying rotations observed with other aliphatic hydroxy-acids, such as lactic (Wislicenus), gluconic (Tollens), glyceric (Frankland), and malic (Walden), which are due to strongly active anhydrides.

d-Acid.

In the resolution of an inactive acid by an alkaloid, it is often difficult, after removal of the alkaloidal salt of one of the active forms, to isolate the other salt from the mother liquors. Should it be possible, however, in the case of the inactive acid to find a metallic salt which is a *dl*-mixture, and not an *r*-compound, and which can be readily separated from its solutions by crystallisation, the resolution may be conveniently carried out (compare Marckwald, *Ber.*, 1899, 32, 1089). This could not be done in the present instance, as the majority of the metallic salts of the β -hydroxybutyric acid do not lend themselves to separation from their solutions by crystallisation. The silver and cadmium salts were the only two which appeared likely to be suitable, but it was found, by aid of the well-known criterion of Bakhuis-Roozeboom for distinguishing between a solid *dl*-conglomerate and a solid *r*-compound, that those salts are *r*-compounds and not *dl*-mixtures.

Recourse was therefore had to the alkaloidal method. It was found that the *d*-acid-*l*-strychnine salt is less soluble in ethyl acetate than the *l*-acid-*l*-strychnine salt, and the former salt was finally separated, the crystallisation being conducted at first from alcohol and finally from ethyl acetate.

From the acid with $[\alpha]_D^{16} + 2.5^\circ$ ($c = 17.056$), recovered from the quinine mother liquors, the strychnine salt was prepared by digestion with the base in ethyl alcohol solution and allowed to crystallise at the temperature of the laboratory for several weeks. The resulting crystalline magma was drained on porous plates and the product (35 grams) repeatedly crystallised from anhydrous ethyl acetate. The progress of the resolution was observed by decomposing the filtrates from each successive crystallisation and noting the activity of the acid. In solutions of similar concentration, the following values for $[\alpha]_D$ were thus obtained: $+9.6^\circ$, 10.2° , 13.0° , 14.0° , 19.7° , 22.3° , 23.1° , and 24.4° . As the $[\alpha]_D$ of the *l*-acid has been found to be 24.9° , the strychnine salt was therefore pure after eight crystallisations. It crystallises from ethyl acetate in glassy, rectangular prisms grouped in rosettes, and a solution of the air-dried salt in ethyl alcohol gave $[\alpha]_D^{23} - 33.8^\circ$ ($c = 1.256$). The salt begins to decompose at 250° , so that melting point determinations were of no aid as an indication of the progress of the resolution.

1406 MCKENZIE: THE RESOLUTION OF β -HYDROXYBUTYRIC

The salt was dissolved in water, in which it suffers considerable hydrolytic dissociation, and the acid was prepared from it by the method already described for the *l*-isomeride. The resulting syrup crystallised immediately on addition of a nucleus of the *l*-acid. The specific rotation, where the concentration of the dried solid was determined by direct weighing, gave a value agreeing within the limit of experimental error with that of the *l*-acid, thus:

$$l = 2, c = 2.226, \alpha_D^{10} + 1.08^\circ, [\alpha]_D^{10} + 24.3^\circ.$$

On analysis, 0.4452 dissolved in water required 42.5 c.c. *N*/10 potassium hydroxide solution for neutralisation. $C_4H_8O_3$ requires 42.8 c.c. for the same amount.

The specific rotation of the potassium salt agreed with that of the potassium *l*-salt.

The *d*-acid-*l*-quinine salt crystallises from water in silky, radiating needles. The air-dried salt contains $1H_2O$ which is lost at 100° :

0.3717, at 100° , lost 0.0153 H_2O . $H_2O = 4.1$.

$C_{20}H_{24}O_2N_2, C_4H_8O_3, H_2O$ requires $H_2O = 4.0$ per cent.

The hydrated salt melts at $108-114^\circ$, the anhydrous salt at $126.5-127.5^\circ$. The air-dried salt dissolved in ethyl alcohol gave:

$$l = 2, c = 3.1100, \alpha_D^{17} - 7.85^\circ, [\alpha]_D^{17} - 126.2^\circ,$$

a value only slightly lower than that of the *l*-acid-*l*-quinine salt.

Both active acids in the form of syrups may be kept for months in a partial vacuum over sulphuric acid without crystallising. A crystalline nucleus of the *l*-acid causes immediate crystallisation, not only of the *l*-syrup, but also of the *d*-syrup; similarly, a nucleus of the *d*-acid causes crystallisation of the *d*-syrup and of the *l*-syrup. The two active nuclei behave, in fact, as if their crystalline forms are identical, and this is not what would be expected if the idea, originally expressed by Pasteur, is universally correct, namely, that isomerides which in solution exhibit opposite signs of rotation are hemihedric when in the crystalline form.

In the light of the observations just mentioned, it was *a priori* unlikely that an active nucleus would cause crystallisation of the inactive syrup, a conclusion borne out by experiments on the pure syrup and its concentrated aqueous solutions at temperatures varying from 30° to that of boiling liquid air. In this connection, the following experiment may also be mentioned. Diacetylracemic anhydride, prepared by acetylating racemic acid by Thiele's method, was obtained as a syrup which, when kept for several hours, did not crystallise until a crystalline nucleus of *d*-diacetylracemic anhydride was introduced. Crystallisation then gradually proceeded and the solid, withdrawn

long before the crystallisation was complete, proved to be inactive. Comparison may also be made with experiments on mandelic acid (McKenzie, *Trans.*, 1899, 75, 964) where the addition of an active nucleus to a supersaturated solution of inactive substance yielded inactive products in every case. These negative results were attributed to the possibility of hemihedrisism being either absent or masked in the active nuclei employed; the *d*-nucleus had the same effect as the *l*-nucleus, the two forms inducing crystallisation as if they were identical in crystalline form.

Separation of an optically active product by sowing an active nucleus either into supersaturated solutions or into superfused liquids of the inactive form is quite exceptional, the only cases on record being the experiments of Gernez with sodium ammonium racemate (*Compt. rend.*, 1866, 63, 843), of Purdie with zinc ammonium lactate (*Trans.*, 1893, 63, 1143; Purdie and Wallace Walker, *Trans.*, 1895, 67, 616), and of Ruff with the benzylphenylhydrazones of erythrose (*Ber.*, 1901, 34, 1362). In the first of these cases, it is known that the hemihedrisism of the active forms is such as is evident from crystal measurement alone; in the two latter cases, the crystals have not been measured.

The following polarimetric observations were made with aqueous solutions of salts of the synthetical *l*-acid :

	<i>c.</i>	<i>t.</i>	<i>l.</i>	α_D	$[\alpha]_D$	$[M]_D$
<i>Sodium salt</i>	8.518	15°	4	-4.93°	-14.5°	-18.3°
	3.4072	17	„	1.95	14.3	18.0
	1.8629	„	„	0.75	13.8	17.4
<i>Potassium salt</i>	4.3742	12	2	1.08	12.3	17.5
	1.7497	„	4	0.83	11.9	16.9
<i>Zinc salt</i>	22.2125	18	2	7.97	17.9	24.3
	8.885	„	„	2.93	16.5	22.4
	3.554	„	4	2.17	15.3	20.8
	1.4216	„	„	0.81	14.2	19.3
<i>Magnesium salt</i>	14.3937	13	2	5.15	17.9	20.6
	5.7575	„	„	1.86	16.2	18.7
	2.303	„	4	1.41	15.3	17.6

With aliphatic hydroxy-acids generally, the molecular rotation of the acids varies considerably with concentration and the values for dilute aqueous solutions of the normal salts are very much higher than those for the free acid. The rotations of β -hydroxybutyric acid and its salts are exceptional in this respect. It has already been mentioned that change of concentration has little influence on the value for the specific rotation of the acid, and it will be seen from the

1408 MCKENZIE: THE RESOLUTION OF β -HYDROXYBUTYRIC

above figures that the salts rotate in the same direction as the acid but to a less degree. There is here no indication of the disturbing effect which the hydroxyl group in other cases is known to exert on optical activity. Consideration of the rotations quoted led to the conclusion that the optical activity of the anhydride of β -hydroxybutyric acid cannot differ much from that of the acid. The following experiments proved this to be the case.

When inactive β -hydroxybutyric acid is heated at 100° , it is partially converted into an anhydride. In one experiment, for example, where the inactive acid was heated for 30 hours at 100° , the behaviour of the resulting syrup on titration with alkali was similar to that observed by Wislicenus with lactic acid (*Annalen*, 1872, 164, 181); the amount of alkali required for neutralisation in the cold was very much less than what would have been required on the assumption that the syrup consisted of the acid alone, whilst the solution, which in the cold had been made feebly alkaline, became acid in reaction, gradually at ordinary temperature, and quickly on boiling. When the *l*-acid is heated at 100° , it suffers partial conversion into an anhydride, which, unlike the anhydrides of lactic and glyceric acids, for instance, rotates in the same direction as the parent acid, but to a less extent. No crotonic acid was observed to be produced at this temperature. 1.1516 grams of the *l*-acid, at 100° for 21 hours, lost 0.0354 gram, and the product, when dissolved in cold water, gave a value for $[\alpha]_D$ about 4° below that of the acid:

$$l = 2, c = 4.4648, \alpha_D^{18} - 1.87^\circ, [\alpha]_D^{18} - 20.9^\circ.$$

The anhydride present was estimated by the method used by Wislicenus for lactic acid. In 1.07 grams of the product, the quantity of acid was found to be 0.40 gram, and of anhydride 0.68 gram, on the assumption that the anhydride has the formula

The constancy of the rotation of the active β -hydroxybutyric acids in aqueous solutions is thus understood. An aqueous solution of the acid may be regarded as containing some anhydride due to the "autocatalysis" brought about by the free acid present (Henry, *Zeit. physikal. Chem.*, 1892, 10, 96), but as the rotation of the anhydride differs so little from that of the acid, a small amount of the former present in the solution cannot be detected by polarimetric observation.

In marked contrast to the corresponding salts of active lactic acid, the rotations of the sodium and potassium salts are little influenced by

* It should be mentioned that the heating of the *l*-acid at 100° does not cause partial racemisation. The product, recovered from the potassium salt remaining from the preceding estimation, had the rotation of the pure *l*-acid.

changes of temperature and concentration. As usual, the Landolt-Oudemans law holds good. The ionic molecular rotation cannot be deduced from observations on the acid, which is not sufficiently ionised in aqueous solutions having a rotatory power large enough in a 400 mm. tube to permit of determinations from which conclusions might be drawn. The ionic molecular rotation, deduced from the determinations on the salts, has a value of about 17° . The zinc salt shows a distinct variation with concentration, the molecular rotatory power falling with dilution. This can be attributed to the low degree of electrolytic dissociation which zinc salts are known to exhibit. A notable instance of the influence of this factor on optical activity is afforded by the observation of Purdie and Irvine (Trans., 1901, 79, 962) on the zinc salt of *d*-dimethoxysuccinic acid, the $[M]_D^{20}$ of which varies from -14.4° ($c = 4.3690$) to $+88.4^\circ$ ($c = 0.5461$).

Partial Resolution of β -Hydroxybutyric Acid in the Living Organism.

The first reference to the fate of β -hydroxybutyric acid in the living organism is by Albertoni (*Arch. exp. Path. Pharm.*, 1884, 18, 238), who did not detect acetoacetic acid as a decomposition product. Minkowski (*Arch. exp. Path. Pharm.*, 1893, 31, 182) found in the case of a dog which had been made diabetic by extirpation of the pancreas and then fed *per os* with sodium *l*- β -hydroxybutyrate, that the urine gave a strong ferric chloride reaction and likewise the Lieben iodoform test. From this research and from that of Araki (*Zeit. physiol. Chem.*, 1894, 18, 1), it was established that β -hydroxybutyric acid is converted in the organism into acetoacetic acid and acetone (compare also Waldvogel, *Centr. inn. Med.*, 1898, 33, 845; Ahrens, *Inaug. Dissert. Göttingen*, 1899). With the view of throwing some light on diabetic coma, Sternberg (*Virchow's Archiv*, 1898, 152, 207) examined the action of inactive sodium β -hydroxybutyrate by injecting this salt into the jugular vein of frogs and cats. He was unable to find any β -hydroxybutyric acid in the urine subsequently examined. Likewise, when the acid was taken into the human system *per os*, the presence of acetone or acetoacetic acid could not be detected. Sternberg concludes that the acid was entirely oxidised.

When an inactive resolvable substance is not entirely oxidised in the organism and can be partly recovered from the urine, it seemed probable, in the light of the experiments of Marckwald and McKenzie on the fractional esterification and hydrolysis of stereoisomerides (*Ber.*, 1899, 32, 2130; 1900, 33, 208; 1901, 34, 469), that a partial resolution of inactive substance would occur under those conditions. This view received a certain support from Brion's observation that *l*-tartaric acid is more rapidly oxidised in the organism than *d*-tartaric acid

1410 McKENZIE: THE RESOLUTION OF β -HYDROXYBUTYRIC

(*Zeit. physiol. Chem.*, 1898, 25, 283), although in Brion's experiments the problem becomes somewhat complicated from the fact that bacterial influence must be considered, the acids having been given *per os*. Neuberg and Wohlgemuth, on the other hand, have recently found (*Zeit. physiol. Chem.*, 1902, 35, 41) that when *r*-arabinose is injected into rabbits, either subcutaneously or intravenously (when bacteria can have no influence in resolving the sugar into its active components), a product consisting of *r*- and *d*-arabinose is recovered in the urine. The same investigators have also observed that *l*-arabinose is more readily attacked in the organism than *d*-arabinose.

From two separate experiments in which inactive potassium and sodium β -hydroxybutyrates respectively were injected subcutaneously into dogs, I have found that (1) acetoacetic acid and acetone were present, (2) a small portion of the β -hydroxybutyrates was not attacked, (3) this product was levorotatory. This result indicates that *d*- β -hydroxybutyrate is more readily decomposed by the organism than the *l*-isomeride. Twenty-three c.c. of an aqueous solution containing 9 grams of inactive potassium β -hydroxybutyrate were injected subcutaneously into a dog, the urine of which, passed during the 24 hours previous to the injection, had been examined by Magnus-Levy's method and found to contain no β -hydroxybutyric acid. Three hundred and fifty c.c. of urine were collected during the next 36 hours, and in this any bacterial growth had been prevented by the presence of thymol. Ammonium sulphate was added and the product evaporated to a small bulk and filtered. After addition of sulphuric acid, the solution was extracted with ether in a continuous extraction apparatus. Water was added to the residue from which the ether had been evaporated and the solution was filtered from a trace of hippuric acid. The filtrate was next distilled in steam until 100 c.c. of the distillate required less than 0.2 c.c. of *N* potassium hydroxide solution for neutralisation. Practically no volatile fatty acids were present. The residue, which was strongly acid to neutral litmus paper but did not turn congo-red paper blue, was neutralised by 6.1 c.c. of *N* potassium hydroxide solution. The solution of potassium salt, decolorised as far as possible by animal charcoal and evaporated to 12 c.c., gave $\alpha_D^{20} - 0.38^\circ$ ($l=2$), whence $[\alpha]_D - 2.6^\circ$. 0.9 Gram of salt was recovered from the 9 grams used. The acid obtained from the salt was also distinctly levorotatory.

In a second experiment, made with 7 grams of inactive sodium salt, similar results were obtained. The urine collected after the injection gave a coloration with ferric chloride solution and also the Lieben iodoform test and a little β -hydroxybutyric acid with a *l*eo-rotation was isolated from it. The urine before the injection did not contain acetoacetic acid, acetone, or β -hydroxybutyric acid. The solution of

recovered β -hydroxybutyric acid gave $\alpha_D^{18^\circ} - 0.16^\circ$ ($l=2$) and this was converted into sodium salt, which had the rotation $\alpha_D^{19^\circ} - 0.12^\circ$ ($l=2$). The sodium salt recovered amounted to 0.8 gram.

As the injections were made subcutaneously, the partial resolution of the acid into its optically active components cannot be ascribed to the action of bacteria, and does not fall under the head of either of Pasteur's methods for resolving inactive substances. The author's thanks are due to Dr. Allan Macfadyen for performing the injections.

The following hypothesis may be advanced to account for the partial resolution described. The *d*-constituent of the inactive substance combines in the organism with an asymmetric complex (*C*), the *l*-constituent combines with the same asymmetric complex, but the rate of combination can in each case be different. On the assumption, firstly, that the combination of the inactive acid with *C* is incomplete, and that the two products (*d*-acid+*C*) and (*l*-acid+*C*) are entirely oxidised, it is obvious that an optically active acid may be obtained. On the assumption, on the other hand, that the combination of the inactive acid with *C* is complete, and that the two products (*d*-acid+*C*) and (*l*-acid+*C*) are not entirely oxidised, the oxidation of those products can proceed at different rates, as they are not "Spiegelbilder." Unequal amounts of (*d*-acid+*C*) and (*l*-acid+*C*) may thus be formed and, by elimination of *C*, the resulting mixture may contain the *r*- (or *dl*-) acid with an excess of the one active stereoisomeride. Other possibilities, for example, where the combination of the inactive acid with *C* is incomplete, and where (*d*-acid+*C*) and (*l*-acid+*C*) are not entirely oxidised, are more complex and need not be discussed.

In order to show that a partial resolution of the acid is possible where the asymmetric complex (*C*) is a substance of known constitution, experiments with *l*-menthol and *l*-borneol were made (compare Marekwald and McKenzie, *loc. cit.*). On account of the tendency of β -hydroxybutyric acid to pass into crotonic acid, the temperature conditions under which the esterifications were conducted had to be kept rather low. The amount of esterification was accordingly slight, but the partial resolution was nevertheless quite marked.

14.6 Grams (1 mol.) of inactive β -hydroxybutyric acid were heated with 21.9 grams (1 mol.) of *l*-menthol at 100° for 8 hours. The product was dissolved in ether, and the unesterified acid removed by extraction with an aqueous solution of sodium carbonate. The solution of the sodium salt was completely freed from menthol by extracting it with ether, and then concentrating by evaporation. It was then acidified and extracted with ether. The resulting acid was made up to 25 c.c. with water, and then gave $\alpha_D^{18^\circ} - 0.08^\circ$ ($l=4$). The mixture of esters remaining after the removal of the unesterified acid was hydrolysed by alcoholic potash, and, after complete removal of

menthol, the acid was obtained as before. Its aqueous solution, made up to 25 c.c., gave $\alpha_D^{18} + 0.05^\circ$ ($l=4$). The total acid recovered was found by titration to be 13.2 grams, of which only 1.1 grams had been esterified.

The inference from this experiment is that, of the two active acids, the *d*-isomeride is the more quickly esterified by menthol. When 14.6 grams of the inactive acid were heated with 21.9 of menthol for 3 hours at 130° , 12 grams of unesterified acid were recovered, and this, when made up to 25 c.c. with water, gave $\alpha_D^{18} - 0.11^\circ$ ($l=4$). On the other hand, the acid obtained by hydrolysing the mixture of menthyl esters, instead of being dextrorotatory, was practically inactive, a phenomenon which can be ascribed to partial racemisation, the *d*-acid-*l*-menthyl ester and the *l*-acid-*l*-menthyl ester being racemised at different rates.

When 8.5 grams of inactive acid were heated with 12.6 grams of *l*-borneol at 100° for 10 hours, 6 grams of unesterified acid were recovered. This, when made up to 25 c.c. with water, gave $\alpha_D^{23} - 0.30^\circ$ ($l=4$). The hydrolysis of the esters yielded 1.1 grams of acid, the aqueous solution of which, made up to 25 c.c., gave $\alpha_D^{19} + 0.17^\circ$ ($l=4$).

The work described in this paper was carried out by aid of the Grocers' Company's Research Studentship.

CHEMICAL DEPARTMENT,

JENNER INSTITUTE OF PREVENTIVE MEDICINE,

LONDON, S.W.