CIX. CHOLEIC ACIDS.

V. THE SEPARATION OF OPTICAL ANTIPODES BY MEANS OF CO-ORDINATION COMPOUNDS¹.

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Of the three methods for the separation of optical antipodes from racemic mixtures originated by Pasteur, the mechanical selection of crystals of asymmetrical habit, the formation of chemical compounds, as salts of a racemic acid with an optically active base, and the destruction of one moiety of the racemate by micro-organisms, the second was used by Marckwald and McKenzie [1899, *cf.* Marckwald and Meth, 1905] for resolution of optically active alcohols through their esters. The third method has been extended by the use of extracellular enzymes instead of micro-organisms and Bredig and Fajans [1909] devised a model for asymmetric catalysis on a purely chemical basis. Willstätter [1904] attempted to separate racemic alkaloids by "dyeing" them on wool. The process of dyeing was explained as a salt formation between the dye and the optically active protein of the animal fibre and thus the experiment would fall under Pasteur's second method. Accepting theories which explain dyeing by co-ordinative valencies, we would classify Willstätter's experiment with the present attempts to resolve racemates by means of co-ordination compounds. Successful experiments with optically active dyes have since been reported by Porter and Ihrig [1919, 1923], and Ingersoll and Adams [1922], while Fischgold and Ammon [1931] obtained evidence of optically selective adsorption of mandelic acid in presence of alkaloids on optically inactive adsorbents.

An important step in this direction was made by Windaus *et al.* [1922-23] who studied the addition compounds of digitonin with such alcohols as α -terpineol, *ac*-tetrahydro- β -naphthol or carvomenthol and observed a great difference in solubility between the *d*-alcohol-digitonide and the *l*-alcohol-digitonide. The utilisation of this property enabled them to effect an almost complete resolution of *dl*- α -terpineol and *dl-ac*-tetrahydro- β -naphthol into active components.

In the course of our studies on choleic acids, we tried the synthesis of addition compounds of desoxycholic acid with racemates representing various types of compounds, such as hydrocarbons, alcohols, ketones and acids.

¹ A report on these experiments was read before the Division of Organic Chemistry at the Annual Meeting of the American Chemical Society at Buffalo, September 2, 1931.

A preliminary report on these experiments was given in 1931¹. Methyl-ethylacetic acid was chosen as the simplest aliphatic monocarboxylic acid with an asymmetrical carbon atom. Methyl-ethylacetic acid-choleic acid obtained by direct synthesis of its constituents contained an excess of $\frac{1}{6}$ of the laevo- over the dextro-form of the fatty acid. No choleic acid could be obtained from mandelic acid. Smaller yet definite preference in the formation of co-ordination compounds was given by the laevorotatory forms of phenyl-ethylethanol and dipentene. Benzoin would not form a choleic acid co-ordination compound under the conditions of our experiments. Two choleic acids, containing one and two molecules of bile acid for each molecule of the ketone, have been described by Rheinboldt *et al.* [1929] for natural *d*-camphor. In the case of synthetic *dl*-camphor a dicholeic acid was obtained, which contained an excess of $\frac{1}{3}$ of the laevorotatory camphor. The laevo-form gives a choleic acid less soluble and presumably more stable than its enantiomorph. In summarising these experiments, one observes that the bile acid, which is dextrorotatory, prefers the laevo-form of each pair. Digitonin, a laevorotatory substance, formed more insoluble compounds with *l*- α -terpineol and *l*-acetetrahydro- β -naphthol [Windaus *et al.*, 1922-23]. Thus, no rule for the formation of co-ordination compounds can be based on the sense of rotation of the components. However, the difference in behaviour of optical antipodes towards natural products like bile acids or saponins helps in classifying one of a pair as the physiological enantiomorph.

While this method is not intended to replace the traditional methods of resolution in their established sphere, it widens the range of resolvable racemates. A study of the most suitable concentrations and solvents will without doubt guide us towards a practical procedure in the preparative employment of this principle, which applies to a great variety of substances. They comprise many groups of relatively inert substances unfit for salt or ester formation, *e.g.* hydrocarbons. But apart from its preparative merits, this method will aid in the decision whether the incompletely known structure of some inactive substance contains an asymmetric centre or not.

Bile salts are known to influence the action of lipolytic enzymes. Thus the optical selectivity of bile acids may play a part in the optical selectivity of esterases and lipases, a phenomenon discovered by Dakin [1903, 1904, 1905, *cf.* Fischer and Bergell, 1903, Kuhn, 1925]. Moreover, the modern theory of enzyme action has advanced to a point where certain groups of the substrates are recognised as responsible for the affinity between enzyme and substrate [Balls, 1930; Waldschmidt-Leitz, 1931]. The optical selectivity of bile acids, together with some of their other features such as structural selectivity [Sobotka and Goldberg, 1932, Part III] and affinity for certain indicators, suggests them as models for the mode of attachment of lipolytic enzymes to lipid substances.

¹ Since this publication, Weiss and Abeles [1931-32] reported the isolation of *l*-*sec*.butyl picramide from *dl*-*sec*.butyl picramide by formation of a co-ordination compound with *d*- β -naphthylcamphylamine.

EXPERIMENTAL PART.

Methylethylacetic acid-choleic acid. 5 g. purest desoxycholic acid, prepared according to Sobotka and Goldberg [1932, Part II], were dissolved in 20 cc. pure inactive methylethylacetic acid and kept in the ice-chest for 24 hours. A crop of 5 g. of crystals was separated. The dried substance showed an acid equivalent of 294–297, while 295.5 is the theoretical value for a dicholeic acid of a valeric acid. The crystals were digested with hot benzene and the benzene treatment repeated two or three times. The combined filtrates were distilled and left a small yellowish glassy residue of bile acid. The fraction of the distillate consisting of methylethylacetic acid was dissolved in the receiving flask with absolute alcohol and made up to 5 cc. It showed $\alpha_D = -0.09^\circ$ in a 2 dm. tube. In another experiment with similar proportions, -0.10° was the rotation of this fraction.

It was possible to recover a greater excess of *d*-methylethylacetic acid from the mother-liquor than of *l*-acid from the choleic acid. The filtrate, after removal of the crystals in the above experiment, was distilled. The distillate had $\alpha_D = +0.2^\circ$ at 27° in a 2 dm. tube. This corresponds to an excess of 0.58 % *d*-methylethylacetic acid of $[\alpha]_D = +17.5^\circ$ or 93 mg. for the total distillate of 16 cc. As 5 g. methylethylacetic-dicholeic acid contains 575 mg. methylethylacetic acid, the excess of *d*-form was $\frac{1}{3}$ of the amount that could be left over, if the desoxycholic acid combined with the *l*-form exclusively.

Phenylethylethanol-choleic acid. 7 g. pure desoxycholic acid were dissolved in 20 g. phenylethylethanol (C_6H_5) (C_2H_5) CH. CH_2 OH. Transparent crystals appeared on slow cooling. They were separated from the mother-liquor and decomposed with benzene. The benzene solution, separated from the insoluble benzene-choleic acid, was expected to contain the phenylethylethanol that had combined with the bile acid. It was distilled under reduced pressure leaving behind small amounts of bile acid that had passed into the benzene solution. The higher boiling fraction of the distillate, made up to 5 cc. in absolute alcohol, showed a rotation of -0.20° in a 2 dm. tube. Assuming the $[\alpha]_D$ of *l*-phenylethylethanol to be 37.6° in absolute alcohol [Levene, Mikeska and Passoth, 1930], this rotation would signify an excess of 13.5 mg. *l*-form in the entire amount of phenylethylethanol recovered from 6 g. choleic acid. The undiluted mother-liquor, freed from dissolved bile acid by vacuum distillation, showed slight dextrorotation ($+0.05^\circ$, $l = 2$ dm.) corresponding to a concentration of 0.14 % excess *d*-form, the $[\alpha]_D$ of phenylethylethanol without solvent being 18.9° . This excess corresponds to 21 mg. for a total of 15 cc. of mother-liquor recovered.

Dipentene-choleic acid. 6 cc. of dipentene of $n_D^{20} = 1.4750$, prepared from terpin hydrate, by boiling it for 10 hours with three equivalents of acetic anhydride according to Ginsberg [1897], were dissolved in 110 cc. hot absolute alcohol containing 24 g. desoxycholic acid. 11.4 g. of crystallised dipentene-choleic acid was obtained on cooling, m.p. 192° (corr.).

The probable co-ordination number is 3 as the acid equivalent was 444,

and the theoretical value for a tricholeic acid $C_{10}H_{16} \cdot 3C_{24}H_{40}O_4$ is 438, while $C_{10}H_{16} \cdot 2C_{24}H_{40}O_4$ demands 460.

In this instance, dissociation of the choleic acid was accomplished by treating the 11 g. of dried crystals with 25 cc. *N* NaOH and extracting the hydrocarbon with ether. The high-boiling fraction of the ethereal extract, made up to 5 cc. with absolute alcohol, had $\alpha_D = -0.08^\circ$ in a 2 dm. tube. This means an excess of *l*-limonene over *d*-limonene of $1\frac{2}{3}$ mg. as the specific rotation of limonene in alcohol is $+120^\circ$. The mother-liquor was evaporated to remove the alcohol, the residue was dissolved in benzene, filtered, and the filtrate distilled. The dipentene fraction of about 4 cc. volume was made up to 5 cc. and had $\alpha_D = +0.09^\circ$ ($l = 2$ dm.). While the rotation matches the rotation observed in the opposite sense with the limonene liberated from its choleic acid, both are exceedingly small in consideration of the high rotation of the limonene antipodes.

Camphor-choleic acid. More encouraging results than in the case of dipentene were obtained with camphor. 2 g. synthetic *dl*-camphor which showed practically no rotation (less than -0.05° in 20 % solution, 2 dm., $[\alpha]_D$ less than -0.12°) and 7 g. desoxycholic acid were dissolved in 12 cc. hot alcohol. The white crystals of camphor-choleic acid, obtained on cooling, melted at 183° (corr.), as does *d*-camphor-choleic acid. The acid equivalent was 478 while the theory requires 468.4 for a camphor-dicholeic acid. Recrystallisation from ethyl alcohol yielded 2.8 g. substance of the same melting-point and acid equivalent 475. It was decomposed by means of hot benzene. 193 mg. of camphor were isolated having $\alpha_D = -0.42^\circ$ in 5 cc. in a 2 dm. tube

$$[\alpha]_D^{23^\circ} = \frac{-0.42^\circ \times 5}{2 \times 0.193} = -5.44^\circ \text{ (absolute alcohol).}$$

Thus the excess of *l*-camphor in the choleic acid was at least $\frac{1}{3}$ of the amount theoretically possible. As 2.8 g. of camphor dicholeic acid contain 446 mg. camphor, there must have been 195 mg. of *d*-camphor and 251 mg. of *l*-camphor.

590 mg. camphor could be isolated by sublimation from the combined benzene filtrates derived from the original mother-liquor

$$[\alpha]_D^{23^\circ} \text{ in alcohol } \frac{+0.41^\circ \times 5}{2 \times 0.590} = +1.74^\circ.$$

The excess of *d*-camphor was about 4 % of the camphor remaining in the mother-liquor.

SUMMARY.

The choleic acid principle can be utilised for the resolution of racemates. Examples of this procedure are given and its bearing on preparative and enzymic problems is discussed.

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