103. *A Note on* cyclo*Hexylideneacetic Acid.*

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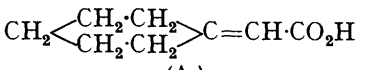
An interest in the configuration of monocyclic *cyclo*hexane derivatives led us during 1935 to attempt—and fail to effect—the optical resolution of *cyclo*hexylideneacetic acid (A), a result which was entirely consistent with the criticisms levelled by Goldschmidt and Gräfinger (*Ber.*, 1935, **68**, 279), Dey and Linstead (J., 1935, 1063), and Desai and Hunter (*Nature*, 1935, **136**, 608) against the claim (Qudrat-i-Khuda, *J. Indian Chem. Soc.*, 1931, **8**, 277; *Nature*, 1933, **132**, 210; 1935, **136**, 301) that isomerides corresponding to the two multiplanar Sachse forms of the simple *cyclo*hexane ring system were stable and capable of isolation.



(A.)

In the course of our work several alkaloidal salts of (A) have been examined. The use of *Aspergillus versicolor* as a resolving agent has been noted, and comparisons recorded with *A. niger* and a mould of the *Penicillium* species concerning the relative efficiencies in destructive attack on the *l*-configuration of lactic acid.

Experimental.

cyclo*Hexylideneacetic Acid*.—This was prepared essentially by Wallach's method (*Annalen*, 1906, **347**, 328; 1909, **365**, 261), which we found to give higher yields than that of Hope and Perkin (J., 1909, **95**, 1363), even when the latter was improved by effecting the formation of the ethyl *cyclo*hexylmalonate under pressure. In an endeavour to find a simpler preparation, the following experiment was performed.

Attempted Perkin Condensation with cyclo*Hexanone*.—A mixture of the ketone (19 g.), fused sodium acetate (10 g.), and acetic anhydride (30 g.) was heated at 180° for 2 days. The mass was then poured while hot into water and neutralised with sodium carbonate, the oily material removed by steam distillation, and the aqueous solution after clarification acidified with hydrochloric acid and left at 0° overnight. No deposition of *cyclo*hexylideneacetic acid occurred. If the oily distillate were repeatedly shaken with fresh sodium bisulphite solution until free from *cyclo*hexanone, a small quantity of a pleasant-smelling liquid remained unattacked; this was taken up in ether, dried, etc., and had b. p. 180—184°. From its analysis (Found: C, 68·2; H, 8·8%) it appeared probable that the substance was the acetate of the enolic form of *cyclo*hexanone (Mannich, *Ber.*, 1906, **39**, 1594) (requiring C, 68·5; H, 8·6%); this was confirmed by (a) the direct production of *cyclo*hexanone on prolonged alkaline hydrolysis, and (b) the isolation of *cyclo*hexanonesemicarbazone, m. p. 166°, from a mixture of the oil (b. p. 180—184°), alcohol, semicarbazide hydrochloride, and dilute caustic soda solution which had been kept at room temperature for a few days.

Attempted Resolution of cyclo*Hexylideneacetic Acid*.—(a) *Brucine*. The alkaloid hydrochloride (1·798 g.; slightly less than ½ equiv.) was dissolved in the minimum amount of water and mixed with a solution of the acid (1·288 g.) in 0·9203N-sodium hydroxide (10 c.c.). The emulsion formed was induced to crystallise by addition of a few drops of alcohol and scratching. The brucine *salt* was thus obtained as white granular crystals, very soluble in alcohol, chloroform, carbon tetrachloride, but sparingly so in light petroleum; m. p. 55—57° (yield, 1·5 g.) (Found: C, 61·6; H, 7·55. $C_{31}H_{46}O_{10}N_2$ requires C, 61·6; H, 7·7%); 0·3935 g., dissolved in 25 c.c. of absolute alcohol, gave α_D — 0·95° (2·2 dcm.), whence $[\alpha]_D^{18}$ *ca.* — 30° (neutral salts of brucine usually show a specific rotation of this order; cf. Tykosiner, *Rec. Trav. chim.*, 1888, **1**, 148).

(b) *Quinine*. Solutions of quinine hydrochloride dihydrate (1·835 g. in water, 30 c.c.) and the sodium salt of (A) (from the acid, 1·835 g., in water, 30 c.c.) were mixed. In various experiments the solutions were either hot or cold, and were in some instances diluted with water and in some with alcohol. The quinine *salt* separated in most cases after long scratching as a matted mass of microcrystalline needles (about 1·8 g.), m. p. 98—104° (Found for a sample dried in a vacuum desiccator: C, 67·1; H, 7·8. $C_{28}H_{36}O_4N_2,2H_2O$ requires C, 67·2; H, 8·0%); 1·8526 g., dissolved in ethyl alcohol (25 c.c.), gave $[\alpha]_{5463}^{18°}$ — 137°. All specimens of the salt and the filtrates from them were shaken repeatedly with aqueous ammonia and chloroform, etc., as usual and from the ammonium *cyclo*hexylideneacetate solutions so obtained the free acid (A) was precipitated by acidification with dilute hydrochloric acid. No activity was observed.

(c) *Strychnine.* After preliminary trials the following procedure was adopted: The sodium salt from 1·1648 g. of the acid and 10 c.c. of 0·8320N-caustic soda, diluted to 500 c.c. with hot water, was treated with strychnine hydrochloride (1·7 g.) in hot water (100 c.c.). After standing at room temperature for 24 hours, the deposited salt (0·2 g.; m. p. 284—285°) was separated, shaken repeatedly with aqueous ammonia and chloroform, and finally with light petroleum. The aqueous solution of the ammonium salt had α_D — 0·08°, acidification (dilute hydrochloric acid) changed this to α_D — 0·07°, but the acid, m. p. 85—90°, obtained by ether extraction had zero rotation. The filtrate from the strychnine salt was evaporated to half bulk; about 0·4 g. of (A) was obtained on cooling, m. p. 90—92° (alone or mixed with an authentic specimen). Finally, concentration of the solution to *ca.* 100 c.c. gave 0·7 g. of long needles, m. p. *ca.* 280—290°, which were too sparingly soluble in alcohol or acetone to permit an α determination; however, decomposition with 2N-sodium hydroxide and chloroform, etc., eventually led to a sodium salt solution which, like the acid obtained by acidification and ether extraction, showed no optical activity.

(d) *Cinchonine and cinchonidine.* The alkaloid hydrochloride (1·483 g.), dissolved in cold water (90 c.c.), alcohol (10 c.c.), and one drop of dilute hydrochloric acid, was mixed with a solution of the acid (1·165 g.) in 0·8320N-sodium hydroxide (10 c.c.). Glassy oils were formed immediately in both instances; these were separated by decantation and rapidly decomposed with aqueous ammonia and chloroform, the aqueous portions being treated similarly at the same time. In neither of the cases did the solutions, or the specimens of acid recovered from them by acidification and ether extraction, exhibit any detectable optical activity.

Action of Moulds on cyclo*Hexylideneacetic Acid and* cyclo*Hexanoneoxime.*—*cyclo*Hexanoneoxime with a *trans* strainless configuration should be an optically resolvable substance. However, all attempts—including the duplication of the methods successfully used for the resolution of camphoroxime (Pope, J., 1899, **75**, 1108; Proc., 1899, **15**, 199)—gave only non-crystallisable gums. We therefore sought to demonstrate the resolvability of the acid and the oxime, and to this end examined the growth of the moulds, *Aspergillus niger*, *A. versicolor*, and a green species of *Penicillium* on aqueous solutions containing these substances as a source of carbon. The experiments were carried out generally as follows: A stock culture solution was made up to the following recipe: water (1 l.), sodium nitrate (2 g.), potassium dihydrogen phosphate (1 g.), potassium chloride (0·5 g.), magnesium sulphate heptahydrate (0·5 g.), ferrous sulphate crystals (0·01 g.). 200-C.c. quantities of this solution were placed in 1 l. Roux culture flasks and sterilised by heating at 130° in an autoclave for 1 hour; when they were cold, about 1 g. of the freshly recrystallised acid or oxime was added, and the solution infected with the mould with the usual precautions and left to incubate at 22° for a few days. When a definite growth of mycelia was noticed, a daily polarimetric examination was made. This was easily performed without altering the horizontal position of the flask or disturbing the carpet of mould by withdrawing 10—15 c.c. of the clear lower liquid through a suitably bent sterilised pipette, stirring it with a little charcoal, and filtering it directly into the polarimeter tube. Growth occurred slowly with the acid, but no perceptible resolution was observed. The oxime appeared to be toxic to the moulds; this action was clearly seen by the addition of a specimen to very strongly growing colonies, *e.g.*, those on media containing glycerol as a carbon source; no further extension or sporing took place.

In later experiments with the acid, ammonium acetate (0·1 g.) was added to the stock culture solution, thus bringing its p_H to 4, with advantageous results on the growth of all three moulds. Small rotations (*ca.* 0·02°) were observed with the solutions containing *A. niger* and *A. versicolor*. Such activities cannot be regarded as significant, however, as they could result from the moulds themselves. Good growths of *A. niger* and the *Penicillium* were secured on a glycerol-containing medium; the mycelia and fruiting hyphæ were separated as cleanly as possible, carefully washed with water, and ground with chalk and water. In both cases the altered extracts were slightly dextrorotatory.

Note on the Use of A. Versicolor.—This mould does not appear to have been used for the present purpose, although it commends itself by its apparently omnivorous character (cf. action on paraffin wax; Chibnall and Hopkins, *Biochem. J.*, 1932, **26**, 133). The following investigatory experiment was instituted to estimate its efficiency relative to the other two used in the present work: 12 flasks, each containing 150 c.c. of the previously described stock culture solution (plus ammonium acetate), were divided into four groups. To nine of the flasks sodium lactate (6·5 g.) was added and all 12 were sterilised as above. Three flasks containing sodium lactate and one without were infected with *A. versicolor*; the other two groups of four were similarly infected with the *Penicillium* and *A. niger*. All were placed in an in-

cubator at 22°. Polarimetric examinations were carried out after good sporing had commenced. The observations tabulated were made after the intervals stated; the angles refer to the rotation given by a column of $l = 2{\cdot}2$ dcm.

Time, days after infection	14	28	30	35	39	42	46
A. versicolor	−0·02°	−0·12°	−0·14°	−0·17°	−0·08°	−0·07°	−0·05°
A. niger	—	—	—	−0·02°	−0·03°	—	—
Penicillium	−0·02°	−0·08°	−0·07°	−0·06°	—	—	—

Thus it appears (1) that *A. versicolor* can under these conditions attack the lævo-form of lactic acid * more rapidly than can the other moulds, and (2) that with it simultaneous destruction of both antipodes is not so pronounced as it is with the other two.

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