8. STUDIES IN DETOXICATION

4. THE RESOLUTION OF *dl*-ISOMENTHOL THROUGH ITS CONJUGATION WITH GLUCURONIC ACID IN THE RABBIT

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It has been shown by Williams [1939] that *dl*-menthol could be resolved to give optically pure *d*-menthol by making use of the glucuronic acid detoxication mechanism of the rabbit. In a similar manner *dl*-isomenthol can be resolved to give optically pure *d*-isomenthol. *l*-isoMenthol can also be obtained but the most laevorotatory fraction was no more than 84 % optically pure. The resolution of *dl*-isomenthol is of special interest in that it has not been done before. Chemical methods, which can be used for the resolution of *dl*-menthol, fail when applied to *dl*-isomenthol mainly because of mixed crystal formation. Read *et al.* [1933] state, "No optical resolution of *dl*-isomenthol has yet been recorded and we now show that neither of the methods recently devised for the resolution of *dl*-menthol [Read & Grubb, 1931; 1933] is applicable to *dl*-isomenthol".

When *dl*-isomenthol is fed to rabbits, 55 % of it is excreted in the urine conjugated with glucuronic acid; the isomenthylglucuronide excreted contains about 60 % *d*- and 40 % *l*-isomeride [Williams, 1938]. The mixed isomenthylglucuronides were isolated as NH_4 salts. It is this mixture which I have used in the work described here. The mixture, on crystallization from water, deposited homogeneous plates which proved to be ammonium *dl*-isomenthyl-*d*-glucuronate ($[\alpha]_D -56^\circ$ in water). Repeated recrystallization of this salt failed to alter its rotation and it gave *dl*-isomenthol on acid hydrolysis. This salt appears to be a "partially racemic" compound of the Ladenburg type (see Cohen [1924] and compare the (-)menthyl *r*-mandelate of McKenzie [1904]). Acidification of solutions of this salt did not give the *dl*-acid, but an amorphous solid which was mainly impure *d*-isomenthylglucuronide. Two recrystallizations of this solid from water gave pure *d*-isomenthylglucuronide, which yielded *d*-isomenthol quantitatively on hydrolysis. The filtrate from the above amorphous solid deposited fine needles on further acidification. Various fractions of these needles were collected showing rotations varying from $[\alpha]_D -59.7^\circ$ to -65.2° (in alcohol). Recrystallization from water produced only small changes in rotation. The fraction $[\alpha]_D -65.2^\circ$ gave, on hydrolysis, an isomenthol which contained 66 % of the *l*-form. No fraction was obtained which gave a more pure *l*-isomenthol than this. It seems probable that we have here an example of mixed crystal formation. The filtrates after the separation of the needles were therefore extracted with ether. Removal of the ether gave a stiff syrup which, when dissolved in water, failed to deposit any crystalline material. The syrup was therefore hydrolysed and the isomenthol obtained contained 84 % of the *l*-form ($[\alpha]_D -17.6^\circ$ in alcohol). The yield of optically pure *d*-isomenthol obtained by this method did not exceed 5 % of the *dl*-isomenthol fed to the rabbits.

EXPERIMENTAL

Preparation of ammonium dl-isomenthylglucuronate

From 15 g. *dl*-isomenthol fed to rabbits, 12 g. of crude ammonium *iso*-menthylglucuronate were isolated from their urine by the method already described for the isolation of the menthyl derivative [Williams, 1939]. This salt on recrystallization from water gave *ammonium dl-isomenthylglucuronate* as elongated plates (yield 8 g.). The crude filtrate from this salt was not further investigated. The salt was purified by recrystallization from water. The pure salt showed $[\alpha]_D^{25} - 56^\circ$ in water ($c = 1$) which was not altered on recrystallization (Found: C, 51.0; H, 9.0; N, 3.5; H_2O , 7.4%. $(C_{16}H_{27}O_7NH_4)_2 \cdot 3H_2O$ requires C, 51.0; H, 9.1; N, 3.7; H_2O , 7.2%). On hydrolysis with dilute H_2SO_4 this salt gave *dl*-isomenthol, M.P. $53-54^\circ$ ¹ and mixed M.P. 54° and $[\alpha]_D^{25} 0^\circ \pm 1^\circ$ in alcohol ($c = 1.1$ and 2 ; two different specimens). Attempts to resolve this salt by fractional precipitation of its aqueous solution with ammonium sulphate failed to give any appreciable separation.

The separation of d-isomenthylglucuronide

The ammonium *dl*-salt (7 g.) was dissolved in 90 ml. of water at room temperature and 10 ml. of $2N H_2SO_4$ added. After 4 hr., a white translucent waxy solid had separated. This was collected and on drying it gave a hard amorphous mass (2.9 g.) showing $[\alpha]_D - 45.5^\circ$ in alcohol. The rotation of this substance was consistently between -45° and -46° in all the experiments carried out. Two recrystallizations of this substance from 150 and 100 ml. of water respectively, gave pure *d-isomenthylglucuronide* (1.21 g.) as long fine needles, M.P. 125° (mixed M.P. $125-126^\circ$ with an authentic specimen) and showing $[\alpha]_D - 43^\circ$ in alcohol ($c = 1$) (Williams [1938] gives $[\alpha]_D - 43.3^\circ$ in alcohol and M.P. 126°). A further 0.145 g. was obtained from the filtrates at various stages, making the total yield of *d-isomenthylglucuronide* 1.355 g.

The filtrate from the waxy solid above was now treated with 10 ml. of $2N H_2SO_4$ and kept 24 hr. at 0° . The solution was filled with long fine delicate needles (2.2 g.), M.P. 115° and showing $[\alpha]_D^{25} - 61.5^\circ$ in alcohol. These needles, on repeated recrystallization from water acidified with H_2SO_4 showed only small increases in laevorotation. From another resolution, needles showing $[\alpha]_D^{25} - 65.2^\circ$ in alcohol ($c = 1$) were obtained and this figure was the maximum laevorotation obtained in all the resolutions carried out for this work. The latter material gave on hydrolysis an *isomenthol*, M.P. 60° and showing $[\alpha]_D^{25} - 9.1^\circ$ in alcohol ($c = 1$). It therefore contained 67% *l-isomenthol*.

The filtrate from the above needles was further acidified with 10 ml. of $2N H_2SO_4$, but even after standing a week the solution deposited no more crystals. The filtrate was therefore extracted with ether to remove any *iso*-menthylglucuronide remaining in the solution. The ethereal extract, after drying with anhydrous Na_2SO_4 , was evaporated and about 1 g. of a stiff syrup, which eventually set to a glass, was obtained. This syrup yielded no crystalline material after dissolution in water and acidifying. The acidified solution was therefore steam-distilled and 0.4 g. of optically impure *l-isomenthol* (84% *l*.) was obtained which had M.P. $74-76^\circ$ and $[\alpha]_D - 17.6^\circ$ in alcohol ($c = 2$).

¹ All melting points are uncorrected.

Preparation and identification of d-isomenthol from the glucuronide

d-isoMenthylglucuronide (2 g.) obtained from the above resolutions was hydrolysed with 20 ml. 0.5*N* H₂SO₄, the *isomenthol* formed being recovered by a simultaneous steam distillation. The distillate containing the solid *isomenthol* was extracted with light petroleum. The extract on evaporation gave optically pure *d*-*isomenthol* (0.85 g.) in almost theoretical yield. It formed long needles M.P. and mixed M.P. 82–83° and showed $[\alpha]_D^{20} + 25.3^\circ$ in alcohol (*c* = 2). Read & Grubb [1934] give $[\alpha]_D + 25.9^\circ$ and M.P. 82.5°. It was further identified as its 3:5-dinitrobenzoate, which formed long needles M.P. 147° and had mixed M.P. 146° with an authentic specimen, M.P. 145° (Read *et al.* [1933] give M.P. 145°).

SUMMARY

dl-*iso*Menthol has been resolved for the first time, through the *isomenthyl*-glucuronide synthesized in the rabbit, following oral administration of *dl*-*isomenthol*.

The crude ammonium *isomenthyl*glucuronate isolated from the rabbit urine gave, on recrystallization from water, *ammonium dl-isomenthylglucuronate* which appears to be a partially racemic compound of the Ladenburg type. This salt cannot be resolved as such, but on conversion into the free acid, crude *d*-*isomenthyl*glucuronide was formed as a waxy solid. The crude material on recrystallization from water gave the pure crystalline acid identical with an authentic specimen. The glucuronide yielded optically pure *d*-*isomenthol* on hydrolysis. *l*-*iso*Menthol was not isolated in optically pure form, although material of 84 % optical purity was obtained.

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