

into two isomeric substances by means of crystallization. The α -methylhexoside chlorhydrin, m.p. 160°, yields triacetyl 3-acetamido α -methylglucoside (7)² in 60 per cent yield, and a trace of an isomer (8), which has not yet been identified. The other chlorhydrin, m.p. 138°, after similar treatment, yields (7) in 50 per cent yield and an isomer (9), which has not yet been identified, in 20 per cent yield.

A syrupy mixture of α -methylhexoside chlorhydrins has been obtained from 2 : 3-anhydro 4 : 6-benzylidene α -methylmannoside by the method mentioned above, but we have not yet been able to separate the constituents. On treatment of this mixture with ammonia and subsequent acetylation, however, a crystalline product is obtained, from which we have separated (7) in 15 per cent yield and an isomer (10) in 65 per cent yield, which differs from (8) and (9).

Finally, the benzylidene group has been removed from 2-acetyl 3-acetamido 4 : 6-benzylidene α -methylaltroside (3), and the product acetylated to give 2 : 4 : 6-triacetyl 3-acetamido α -methylaltroside, which proves to be identical with (10).

Constants for the derivatives described are appended.

- (1) m.p. 181–182°; $[\alpha]_D^{25} + 51.3^\circ$ in chloroform ($c = 2.13$).
- (2) m.p. 266°; $[\alpha]_D^{25} + 45.6^\circ$ in chloroform ($c = 0.56$).
- (3) m.p. 201°; $[\alpha]_D^{25} + 14.6^\circ$ in chloroform ($c = 2.33$).
- (4) m.p. 235°; $[\alpha]_D^{25} + 45.5^\circ$ in chloroform ($c = 0.85$).
- (5) m.p. 188°; $[\alpha]_D^{25} + 43.4^\circ$ in chloroform ($c = 1.25$).
- (6) m.p. 260°; $[\alpha]_D^{25} + 70.3^\circ$ in chloroform ($c = 0.87$).
- (7) m.p. 179°; $[\alpha]_D^{25} + 105.9^\circ$ in chloroform ($c = 2.12$).
- (8) m.p. 130°; $[\alpha]_D^{25} + 95.7^\circ$ in chloroform ($c = 0.74$).
- (9) 5 group; $[\alpha]_D^{25} + 50.4^\circ$ in chloroform ($c = 2.73$).
- (10) m.p. 177°; $[\alpha]_D^{25} + 34.7^\circ$ in chloroform ($c = 2.38$).

Full details of the above transformations and evidence of identification will be published shortly.

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March 1.

¹ Robertson, Myers and Tetlow, *NATURE*, **142**, 1076 (1938).

² Peat and Wiggins, *J. Chem. Soc.*, 1810 (1938).

³ Robertson and Tetlow, unpublished result.

⁴ Robertson and Dunlop, *J. Chem. Soc.*, 472 (1938).

Use of *dl*-Menthol for the Preparation of Biosynthetic Glucuronic Acid

GLUCURONIC acid, within recent years, has been shown to be a compound of considerable biological importance. Besides playing an important part in the detoxication mechanisms of the body, this carbohydrate derivative is also an essential component of certain immuno-polysaccharides¹. It is found in many mucoproteins² and may be a component of the blood anti-coagulant, heparin³; furthermore, certain sex hormones of human pregnancy urine are found combined with glucuronic acid⁴. Investigations on the chemical nature and biological function of these substances, which will also involve synthetic experiments, will be greatly facilitated if glucuronic acid is obtainable in relatively large amounts. A convenient chemical synthesis of glucuronic acid, such as exists for galacturonic acid⁵, is not available. The main source of it has been biological, advantage being taken of its function in the mammal as a detoxicating agent.

A good method for obtaining biosynthetic glucuronic acid, worked out by Quick⁶, depends upon the

formation and excretion of bornylglucuronic acid following the ingestion of borneol by dogs (under the best conditions the administration of 1 gm. borneol gives 1 gm. zinc bornylglucuronate). In many laboratories, however, it may be inconvenient to keep dogs for long periods, and the collection of urine from dogs fed with borneol requires a large metal-lined cage. Rabbits, however, are much more convenient to use, and an ordinary wire cage set in a suitable funnel can be utilized for collecting the urine.

In a recent paper⁷, I showed that good yields of conjugated glucuronic acid are obtainable from rabbits fed with *dl*-menthol, *dl*-isomenthol and *d*-isomenthol. This investigation has now been extended, and it has been found that *dl*-menthol is the most suitable substance to use when relatively large amounts of glucuronic acid are required. *dl*-Menthol conjugates with glucuronic acid to the extent of 60 per cent in the rabbit, and a higher yield of conjugated glucuronic acid is obtained than in Quick's method using dogs and borneol. *dl*-Menthol (synthetic) can be purchased in large amounts and has no effect on rabbits at the dose level (3 gm.) used. It has a low melting point (34° C.) and can therefore be administered by stomach tube as a partial emulsion in a small amount of warm water. Maximal conjugation with glucuronic acid occurs on using a dose of 3 gm. per rabbit (weighing 2–2.5 kgm.) and excretion of the conjugated product is complete in about 30 hours.

The isolation of menthylglucuronic acid (as the ammonium salt) is a short and easily effected procedure⁷. Ammonium menthylglucuronate is obtained in consistent yields of 1.4 gm. per gm. of *dl*-menthol fed. Thus, on feeding 3 gm. of *dl*-menthol to each of six rabbits, 25 gm. of the ammonium salt was isolated. On suitable treatment with acid, this salt gives glucuronic acid in good yields. The ammonium menthylglucuronate ($[\alpha]_D = ca. -30^\circ$ in water), containing about 60 per cent *d*- and 40 per cent *l*-menthylglucuronic acid, can be resolved by fractional crystallization from water, and the hitherto undescribed *d*-menthyl- β -*d*-glucuronide, $C_{16}H_{28}O_7$, $1\frac{1}{2} H_2O$ (m.p. 110–112° C., $[\alpha]_D = ca. +5^\circ$ in alcohol) can be obtained. *l*-Menthyl- β -*d*-glucuronide has been previously described on several occasions.

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March 8.

¹ *Ann. Review Biochem.*, **6**, 103 (1937).

² See Levene, "Hexosamines and Mucoproteins" (London, 1925).

³ *Ann. Reports Chem. Soc.*, **34**, 294 (1937).

⁴ *Ann. Review Biochem.*, **7**, 273, 279 (1937).

⁵ Sell and Link, *J. Amer. Chem. Soc.*, **60**, 1813 (1938).

⁶ Quick, *J. Biol. Chem.*, **74**, 331 (1927).

⁷ Williams, *Biochem. J.*, **32**, 1849 (1938).

Diaphorase I and II

WE have used the name 'diaphorase' to designate the enzyme which catalyses the transport of hydrogen from dihydrocodehydrogenase I (CoH_2 I) to acceptors like methylene blue or cytochrome, but not to molecular oxygen^{1,2,3}. This enzyme, which had been detected independently by Dewan and Green⁴ ("coenzyme factor"), occurs in all animal tissues so far examined as well as in yeast^{5,6} and in higher