39. STUDIES IN DETOXICATION

5. THE PREPARATION OF *d*-GLUCURONE FROM AMMONIUM MENTHYLGLUCURONATE

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SINCE *d*-glucuronic acid is an essential constituent of many biologically important products (e.g. heparin, certain immuno-polysaccharides and sex hormone glucuronides), its preparation is of the utmost importance. It is, however, still difficult to obtain synthetically on a large scale. Synthetic methods have been devised by Fischer & Piloty [1891], Smolenski [1923], Zervas & Sessler [1933] and recently by Stacey [1939]. These methods are difficult to carry out and, apart from the last, the yields are poor. Yields of 5% of glucuronic acid were obtained by Weinmann [1929] from gum arabic, whilst Kalb & Falkenhausen [1927] prepared it in small yield by oxidation of cellulose.

The best methods for obtaining glucurone (the form in which glucuronic acid is usually isolated) are those depending upon the glucuronic acid detoxication mechanism of mammals. The old method for its large-scale preparation [Thierfelder, 1887; Fischer & Piloty, 1891] was from the conjugated glucuronide, euxanthic acid [cf. Robertson & Waters, 1931] which, as the magnesium salt, is the main constituent of Indian yellow or puree, a pigment obtained from the urine of cows fed on mango leaves. This pigment, however, is no longer available in large quantities. Quick's method [1927], using zinc bornylglucuronate, gives good yields, but after much experience with it [e.g. Pryde & Williams, 1933] I find the method described here to be more convenient [cf. Williams, 1939, 1] and to give much larger yields of glucurone. A method of preparing glucuronic acid from ammonium *l*-menthylglucuronate has been described by Ehrlich & Rehorst [1925].

The success of the biosynthetic method depends on (a) the choice of a suitable glucuronogenic drug which conjugates to a large extent in a conveniently managed animal, (b) an easy method of isolating the conjugated product in good yield from the urine and (c) a suitable method for isolating glucurone from the glucuronide. The method described below appears to satisfy these criteria. Nearly 60% of the glucuronogenic drug (*dl*-menthol) used is excreted by the rabbit conjugated with glucuronic acid [Williams, 1938] and nearly all of the conjugated compound can be isolated from the urine as the ammonium salt. The crude dry ammonium salt contains 85% ammonium menthylglucuronate, 10% ammonium sulphate and 5% water; this salt should therefore yield, on hydrolysing, 42–43% of its weight as glucurone ($C_6H_8O_6$) and 38% as menthol. In actual practice, using the method described below, about 80% of the theoretical amount of glucurone can be recovered on the 50–100 g. scale. The overall yield of crude glucurone is therefore 39–40 g. for every 100 g. of *dl*-menthol fed.

EXPERIMENTAL

Preparation of ammonium menthylglucuronate. Rabbits (2–2.5 kg.) were given 3 g. of *dl*-menthol as a partial emulsion in warm water by stomach tube. The tube was washed down with warm water so that the total water given was about 50 ml. The rabbits were then put in cages placed in tinned-copper funnels and the urine collected for about 30–40 hr. The urine and funnel washings were filtered through muslin and made alkaline with a few drops of ammonia (sp. gr. 0.880), then raised to the boil and quickly filtered¹ to remove precipitated phosphates. Ammonium sulphate (50 g./100 ml. urine) was gradually added to the filtrate with occasional shaking, the solution being simultaneously heated to boiling. The hot solution was immediately filtered¹ to remove a small precipitate of protein² and the filtrate kept overnight at 0°, whereby ammonium menthylglucuronate crystallized and filled the solution. The salt was sucked dry, pressed to remove the last traces of mother liquor and then dried *in vacuo* over CaCl_2 . The yield of dry crude salt was 1.4 g./g. of *dl*-menthol fed. This preparation has been successfully carried out some 30 times during the past 3 years. Table 1 gives the results of five experiments carried out recently. The same yield was obtained from rabbits which had no previous feeding with menthol (or any other compound) as from rabbits which at some time or other had been fed with menthol.

Table 1. *Preparation of ammonium menthylglucuronate*

Exp. no.	No. of rabbits used	Total <i>dl</i> -menthol fed* g.	Time of urine collection hr.	Yield dry NH_4 salt g.	Yield per g. menthol fed g.	$[\alpha]_D$ of 2% aqueous solution
1	8	24	43	34.0	1.42	–27.7°
2	3	9	43	13.4	1.49	–28.3°
3	1	3	19	3.8	1.27	–28.6°
4	8	24	43	32.8	1.37	–28.3°
5	4	12	43	17.1	1.42	–26.3°

* Dose 3 g./rabbit.

The salt dissolved in water to give a clear colourless solution and specimen 1 (see Table 1) contained 4.7% H_2O . Specimens 1 and 2 were analysed for sulphate and contained 6.7 and 7.55% SO_4 respectively or 9.2 and 10.4% calc. as $(\text{NH}_4)_2\text{SO}_4$; neither specimen gave any ash when calcined. The specific rotations quoted in Table 1 show that all five specimens have the same composition. This salt therefore contains about 85% anhydrous ammonium menthylglucuronate ($\text{C}_{16}\text{H}_{27}\text{O}_7\text{NH}_4$) and should yield 43.4% of glucurone ($\text{C}_6\text{H}_8\text{O}_6$).

Preparation of glucurone. A typical preparation was carried out as follows: 50 g. of the above salt were shaken with 400–500 ml. water. Most of the salt dissolved and its solution was complete on adding 100 ml. of 2N H_2SO_4 . The free menthylglucuronic acid was extracted from the solution with six 400 ml. portions of ether and after the sixth extraction the residual aqueous solution gave only a faint naphthoresorcinol reaction. It was not necessary to dry the ethereal extract, in fact other experiments showed that drying, especially with

¹ For quick filtration, Green's 450 grey crepe filter papers were found to be very satisfactory.

² It was stated in Part III [Williams, 1939, 2] that this protein was probably a mucoid. It is, however, mainly denatured albumin. Rabbits receiving a 3 g. dose of *l*- or *dl*-menthol, *d*- or *dl*-isomenthol, *l*-menthone or *d*-neomenthol show a slight albuminuria. Tests on control rabbit urine showed no albuminuria.

anhydr. Na_2SO_4 resulted in a loss of glucuronide owing to considerable adsorption of the latter on the sulphate. The adsorbed glucuronide could not be re-extracted with ether.

The ethereal solution was evaporated under slightly diminished pressure in a 500 ml. distilling flask fitted with a continuous feed arrangement. The residue was a coloured syrup which crystallized completely on cooling. The menthylglucuronide was now dissolved in 200 ml. 0.4 N H_2SO_4 with heating and the solution simultaneously hydrolysed and steam-distilled until no more menthol distilled. In this experiment the menthol was recovered in two fractions thus:

- (1) 4.4 g. showing $[\alpha]_D^{21} + 28.5^\circ$ ($c=2$, in alcohol),
- (2) 13.5 g. showing $[\alpha]_D^{21} + 8.3^\circ$ ($c=2$, in alcohol)

[cf. Williams, 1939, 2]. About 18 g. of menthol were recovered from a possible 19.2 g.

The solution after removal of menthol was treated with a slight excess of "Analar" lead acetate solution (16 g. in 100 ml. water) [cf. Stacey, 1939] and the precipitated lead sulphate was removed by centrifuging. The clear centrifugate and washings were treated with H_2S to precipitate any lead present, as sulphide which was then filtered off under suction. Colloidal PbS was removed by adding a little blood charcoal and filtering. After aeration to eliminate H_2S , the pale yellow clear solution (ca. 500 ml.) was concentrated to a thin syrup at 50° *in vacuo*. This syrup deposited 6.6 g. of almost pure glucurone, M.P. $176\text{--}177^\circ$, on standing at room temperature overnight. These crystals were collected, washed with methyl alcohol and the filtrate again concentrated *in vacuo* to a syrup. The syrup was dissolved in methyl alcohol and the solution deposited 5.6 g. of glucurone. Concentration to a syrup and treatment with methyl alcohol was thrice repeated and the following amounts of glucurone obtained, 2.3, 1.8 and 2.2 g. The last fraction was coloured, but in the first four were white (rotations are recorded in Table 2). A total yield of about 18 g. was therefore obtained from a possible 21.5 g.; 16 g. were obtained in a fairly pure state. This yield corresponds to 39–40 g. glucurone for every 100 g. *dl*-menthol fed. It was further purified by recrystallization from methyl alcohol.

Table 2. *Preparation of glucurone*

Fraction	Yield g.	M.P.*	$[\alpha]_D^{19}$ in 1% aqueous solution*
1	6.6	176–177°	+20.7°
2	5.6		
3	2.3	157°	+19.7°
4	1.8		
5	2.2	132°	+21.0°

* Stacey [1939] gives M.P. $176\text{--}178^\circ$ and $[\alpha]_D + 20^\circ$.

A strong aqueous solution of glucurone was added to a solution of 2:4-dinitrophenylhydrazine in acid (H_2SO_4) alcohol. On standing at room temperature for 30 min. nodular masses of bright yellow micro-needles, which appeared to be the 2:4-dinitrophenylhydrazone of glucuronic acid, separated. The compound was washed with water containing HCl and then with water. It crystallized from hot water as long deep yellow needles, M.P. 205° (decomp.), and from hot alcohol as masses of bright yellow micro-needles, M.P. and mixed M.P. with the deep yellow form, 205° (decomp.). It reduced Fehling's solution on boiling and

dissolved in dilute NaOH giving a deep purplish-red solution which was decolorized on acidifying. (Found: C, 38.7; H, 4.0; N, 15.0%; $C_{12}H_{14}N_4O_{10}$ requires C, 38.5; H, 3.8; N, 15.0%.)

SUMMARY

A new method is described for the preparation of glucurone from ammonium menthylglucuronate isolated from the urine of rabbits fed with *dl*-menthol. A yield of about 39–40 g. of glucurone per 100 g. *dl*-menthol fed was obtained.

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