Loebl, H., Stein, G. & Weiss, J. (1950). *J. chem. Soc.* p. 2704.
Loebl, H., Stein, G. & Weiss, J. (1951). *J. chem. Soc.* p. 405.
Meyer, E. (1905). *Hoppe-Seyl. Z.* **46**, 497.
Neuberg, C. & Welde, E. (1914). *Biochem. Z.* **67**, 18.
Parke, D. V. & Williams, R. T. (1951). *Biochem. J.* **48**, 624.
Robinson, D., Smith, J. N. & Williams, R. T. (1951). *Biochem. J.* **50**, 221.
Sieberg, E. (1914). *Hoppe-Seyl. Z.* **92**, 331.
Smith, J. N., Spencer, B. & Williams, R. T. (1950). *Biochem. J.* **47**, 284.
Smith, J. N. & Williams, R. T. (1949). *Biochem. J.* **44**, 242.
Smith, J. N. & Williams, R. T. (1950). *Biochem. J.* **46**, 243.
Stein, G. & Weiss, J. (1950). *Nature, Lond.*, **166**, 1104.
Young, L. (1950). *Biochem. Soc. Symp.* no. 5, 27.

Studies in Detoxication

41. A STUDY OF THE OPTICAL ROTATIONS OF THE AMIDES AND TRIACETYL METHYL ESTERS OF SOME BIOSYNTHETIC SUBSTITUTED PHENYLGLUCURONIDES

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Smith (1949) observed that the triacetyl methyl ester of β-*o*-cyanophenylglucuronide had an optical rotation in chloroform which was appreciably more negative than the corresponding *m*- and *p*-isomers. We decided, therefore, to prepare, by feeding the necessary phenols to rabbits, a number of such substituted phenylglucuronides and their derivatives to obtain further information on this point. Our results suggest that in the case of the triacetyl methyl esters of the *ortho*-substituted phenylglucuronides we have instances of the phenomenon of restricted rotation which shows itself as an abnormal negative optical rotation. The abnormal positive optical rotations of triacetyl β-*o*-nitrophenyl-D-glucuronide and its methyl ester have already been commented upon (Robinson, Smith & Williams, 1951).

EXPERIMENTAL

General method of preparation of glucuronides

In most cases the glucuronides were isolated as basic Pb salts. Two rabbits (3 kg.) were each given by stomach tube 2 g. or less of the phenol suspended in water. The 24 hr. urine was brought to pH about 4 with a little glacial acetic acid and then treated with saturated aqueous normal lead acetate until precipitation was complete. The precipitate was removed by centrifuging and then discarded. The supernatant fluid was now brought to pH about 8 with a little NH_3 (sp.gr. 0·88) and saturated aqueous basic lead acetate added in excess. The basic lead precipitate was washed on the centrifuge, made into a fine suspension in water and the lead removed by saturation with H_2S. After removal of PbS by filtration, the aqueous solution of the glucuronide was concentrated to a small volume *in vacuo* at 45°. Some glucuronides crystallize out at this stage, whereas others are obtained as water-soluble gums often containing inorganic material. These gums can be purified by dissolution in water and precipitation of the inorganic material by adding ethanol. The precipitate of inorganic material may sometimes contain salts of the glucuronides from which the glucuronide can be freed by careful addition of dilute H_2SO_4 to an ethanolic suspension. The extent to which these salts are formed appears to depend to some extent on the purity of the basic lead acetate used.

Methyl esters of glucuronides were prepared by dissolving the glucuronide gum in methanol and methylating with diazomethane in ether until the gum obtained on evaporating the solvents was neutral. In most instances the methyl esters were not obtained crystalline even when pure crystalline glucuronides were methylated. The methyl esters of glucuronides were found to be somewhat unstable and tended to decompose on boiling in water or drying at 100°.

Crystalline derivatives were usually obtained by acetylating the methyl esters at room temperature overnight with equal volumes of pyridine and acetic anhydride. On pouring the mixture into water, the triacetyl methyl ester of the glucuronide usually separated in crystalline form. Since these triacetyl esters crystallize easily, they are eminently suitable for characterizing glucuronides.

Crystalline glucuronidamides were prepared by dissolving the triacetyl methyl esters in methanol and saturating the solution with dry ammonia. The amides separated from the solution either on keeping or on concentrating. Most of these amides were sparingly soluble compounds of high melting point at which they decompose. Many tend to retain half a molecule of water. In view of their unsatisfactory melting points and sparing solubility, they are not nearly as suitable as the triacetyl methyl esters for characterizing glucuronides.

Melting points are uncorrected; rotations were measured in 2 dm. tubes and when *c* was about 1%, the error in $[\alpha]_D$ was $\pm 1°$ or less. With the sparingly soluble glucuronidamides *c* was usually 0·1 and the error in $[\alpha]_D$ was $\pm 5°$. Compounds were dried at room temperature over $CaCl_2$, unless otherwise stated.

Fluorophenylglucuronides

o-*Derivatives.* Two rabbits each receiving 1 g. of *o*-fluorophenol (b.p. 151–152°/760 mm.) yielded from a 24 hr. urine, 2·7 g. of alcohol-soluble glucuronide gum. On methylation the latter yielded a non-crystalline methyl ester which was acetylated during 20 min. with acetic anhydride (7 ml.) and perchloric acid (2 drops, 60%). The mixture was diluted with water and *triacetyl β-o-fluorophenylglucuronide methyl ester* (2·6 g.) separated and was recrystallized from ethanol. It had m.p. 151–152° and $[\alpha]_D^{23°}$ −44·7° (*c* = 1 in $CHCl_3$). (Found: C, 53·3; H, 5·0. $C_{19}H_{21}O_{10}F$ requires C, 53·3; H, 4·9%.) On treatment with methanolic ammonia *β-o-fluorophenylglucuronidamide*, m.p. 208–210° (decomp.) from ethanol and $[\alpha]_D^{20°}$ −71° (*c* = 0·1 in methanol) was obtained. (Found: C, 50·1; H, 5·0; N, 4·4. $C_{12}H_{14}O_6NF$ requires C, 50·2; H, 4·9; N, 4·9%.)

p-*Derivatives.* The glucuronide gum from the 24 hr. urine of two rabbits which had each received 1 g. of *p*-fluorophenol (b.p. 185°/760 mm.) was prepared. After methylation, the product was acetylated with pyridine and acetic anhydride and yielded *triacetyl β-p-fluorophenylglucuronide methyl ester* (1·4 g.). On recrystallization from ethanol, it formed needles, m.p. 135° and $[\alpha]_D^{25°}$ −33° (*c* = 1 in $CHCl_3$). (Found: C, 53·0; H, 5·1%.) *β-p-Fluorophenylglucuronidamide* was prepared as above and had m.p. 224° (decomp.) and $[\alpha]_D^{20°}$ −77° (*c* = 0·1 in methanol). (Found: C, 49·8; H, 5·0; N, 4·5%.)

Chlorophenylglucuronides

o-*Derivatives.* *o*-Chlorophenol was fed to three rabbits (dose 1·5 g.) and the 24 hr. urine made acid to congo red with HCl. It was then continuously extracted with ether for 7 hr. Evaporation of the ether left a glucuronide gum (0·8 g.) which on methylation and acetylation yielded *triacetyl β-o-chlorophenylglucuronide methyl ester* (0·9 g.). This formed colourless needles from ethanol, m.p. 151–152° and $[\alpha]_D^{20°}$ −65° (*c* = 1 in $CHCl_3$). (Found: C, 51·8; H, 4·9; OMe, 7·2. $C_{19}H_{21}O_{10}Cl$ requires C, 51·3; H, 4·8. OMe, 7·0%.)

p-*Derivatives.* Methylation of *p*-chlorophenylglucuronide (Spencer & Williams, 1950) yielded the *methyl ester* as an oil which crystallized at 0°. On recrystallization from water it formed needles, m.p. 157° and $[\alpha]_D^{26°}$ −83° (*c* = 0·5 in water). (Found: OMe, 11·4. $C_{13}H_{15}O_7Cl$ requires OMe, 10·3%.) On acetylation the ester yielded *triacetyl β-p-chlorophenylglucuronide methyl ester* (needles from aqueous ethanol), m.p. 151–152° and $[\alpha]_D^{24°}$ −32·9° (*c* = 1·26 in $CHCl_3$). (Found: C, 51·5; H, 4·5; OMe, 6·6. $C_{19}H_{21}O_{10}Cl$ requires C, 51·3; H, 4·8; OMe, 7·0%.) Acetylation of *p*-chlorophenylglucuronide (0·5 g.) with acetic anhydride (1 ml.) and a trace of perchloric acid, yielded, on dilution of the acetylation mixture with water, *triacetyl β-p-chlorophenylglucuronic acid* (0·6 g.). This formed leaflets from aqueous methanol, m.p. 175–176° and $[\alpha]_D^{20°}$ −20·8° (*c* = 1·2 in $CHCl_3$). (Found: C, 50·2; H, 4·4. $C_{18}H_{19}O_{10}Cl$ requires C, 50·2; H, 4·4%.)

Bromophenylglucuronides

o-*Derivatives.* The glucuronide gum from the 24 hr. urine of two rabbits which had received 2 g. each of *o*-bromophenol (b.p. 195–198° at 760 mm.) was methylated and acetylated. It yielded 2·2 g. *triacetyl β-o-bromophenylglucuronide methyl ester* as colourless needles, m.p. 141–143° (from ethanol) and $[\alpha]_D^{20°}$ −64·6° (*c* = 1 in $CHCl_3$). (Found: C, 47·0; H, 4·1; CMe, 6·2. $C_{19}H_{21}O_{10}Br$ requires C, 46·6; H, 4·3; OMe, 6·3%.)

β-o-Bromophenylglucuronidamide formed needles from water, m.p. 202–205° (decomp.) and $[\alpha]_D^{20°}$ −63·4° (*c* = 0·4 in water). (Found: C, 40·4; H, 4·2; N, 3·8; Br, 21·4. $C_{12}H_{14}O_6NBr$, 0·5 H_2O requires C, 40·4; H, 4·2; N, 3·9; Br, 22·4%.)

m-*Derivatives.* From 2·8 g. of *m*-bromophenol (b.p. 264–266° at 760 mm.) fed to two rabbits, there was similarly obtained 2·1 g. of *triacetyl β-m-bromophenylglucuronide methyl ester*, as needles from ethanol, m.p. 121° and $[\alpha]_D^{20°}$ −33·1° (*c* = 1 in $CHCl_3$). (Found: C, 46·9; H, 4·4; OMe, 6·1%.) *β-m-Bromophenylglucuronidamide* formed colourless needles of the monohydrate from water, m.p. 215–218° (decomp.) and $[\alpha]_D^{20°}$ −57° (*c* = 0·2 in water). (Found: C, 39·6; H, 4·5; N, 4·1; Br, 21·1. $C_{12}H_{14}O_6NBr$, H_2O requires C, 39·4; H, 4·4; N, 3·8; Br, 21·8%.)

p-*Derivatives.* A total of 3·5 g. of *p*-bromophenol was fed to two rabbits and the urine was worked up after 4 hr. The glucuronide gum in this case crystallized and 1·2 g. of *β-p-bromophenylglucuronide* was obtained as colourless needles (from water) of the *dihydrate*, m.p. 157° and $[\alpha]_D^{20°}$ −68·2° (*c* = 1 in water). (Found: C, 37·5; H, 4·4; Br, 19·7. $C_{12}H_{13}O_7Br$, $2H_2O$ requires C, 37·4; H, 4·4; Br, 20·7%.) From the urine of the next 20 hr., the glucuronide gum was prepared, methylated and acetylated to give 1·2 g. of *triacetyl β-p-bromophenylglucuronide methyl ester* (needles from ethanol) m.p. 157–158° and $[\alpha]_D^{20°}$ −28° (*c* = 1 in $CHCl_3$). (Found: C, 47·0; H, 4·5; OMe, 6·1%.) *β-p-Bromophenylglucuronidamide* formed colourless needles from water, m.p. 255–265° (decomp.) and $[\alpha]_D^{20°}$ −61° (*c* = 0·05 in water). (Found: C, 41·3; H, 4·0; N, 4·7. $C_{12}H_{14}O_6NBr$ requires C, 41·4; H, 4·1; N, 4·0%.)

Iodophenylglucuronides

o-*Derivatives.* After feeding 6 g. *o*-iodophenol (m.p. 43°) 4·71 g. of purified glucuronide gum was obtained. The gum (1 g.) in a little water was neutralized with the calculated amount of Na_2CO_3, and on evaporation *sodium β-o-iodophenylglucuronate* was obtained as white needles with $[\alpha]_D^{20°}$ −59° (*c* = 1 in water). (Found: C, 34·6; H, 3·2; I, 32·7; Na, 5·5. $C_{12}H_{12}O_7INa$ requires C, 34·5; H, 2·9; I, 30·4; Na, 5·5%.) The rest of the gum was converted into the gummy methyl ester (2·22 g.) which on acetylation yielded 1·9 g. of *triacetyl β-o-iodophenylglucuronide methyl ester*, m.p. 161–162° (from methanol) and $[\alpha]_D^{20°}$ −63·3° (*c* = 1 in $CHCl_3$). (Found: C, 42·7; H, 4·3; $C_{19}H_{21}O_{10}I$ requires C, 42·6; H, 4·0%.)

p-*Derivatives.* From the urine of a rabbit which had received 2 g. of *p*-iodophenol (m.p. 92°) there was isolated 1·76 g. of p-*iodophenylglucuronide sesquihydrate*. It formed white needles from water, m.p. 154° and $[\alpha]_D^{20°}$ −68·8° (*c* = 0·4 in water). (Found: C, 34·1; H, 3·8; I, 30·0. $C_{12}H_{13}O_7I$, 1·5 H_2O requires C, 34·1; H, 3·8; I, 29·3%.) On methylation with ethereal diazomethane p-*iodophenylglucuronide methyl ester* was obtained which crystallized with difficulty from water. The ester melted at about 158°; $[\alpha]_D^{20°}$ −68° (*c* = 1 in ethanol). (Found: C, 37·0; H, 4·1; OMe, 7·5. $C_{12}H_{15}O_7I$, 0·5 H_2O requires C, 37·2; H, 3·6; OMe 7·4%.) On acetylation it yielded *triacetyl β-p-iodophenylglucuronide methyl ester*, m.p. 167–168° (from methanol) and $[\alpha]_D^{20°}$ −25·8° (*c* = 1 in $CHCl_3$). (Found: C, 42·5; H, 4·2; OMe, 6·2. $C_{19}H_{21}O_{10}I$ requires C, 42·6; H, 4·0; OMe, 5·8%.)

Cresylglucuronides

o-*Derivatives.* Four rabbits received 1 g. each of *o*-cresol and an 18 hr. urine yielded 6·1 g. of crude glucuronide gum. After methylation and acetylation 0·64 g. of pure *triacetyl β-o-cresylglucuronide methyl ester* was obtained as white needles (from ethanol) m.p. 131° and $[\alpha]_D^{20°}$ −46·7° (*c* = 1 in $CHCl_3$). (Found: C, 56·7; H, 5·7; OMe, 7·2. $C_{20}H_{24}O_{10}$ requires C, 56·6; H, 5·7; OMe, 7·3%.) The residues from the preparation of the triacetyl methyl ester on treatment with ammonia yielded 0·13 g. of *β-o-cresylglucuronidamide*, m.p. 200–202° (from methanol) and $[\alpha]_D^{20°}$ −71·2° (*c* = 0·1 in ethanol). (Found: C, 54·1; H, 6·2; N, 4·8. $C_{13}H_{17}O_6N$, 0·5 H_2O requires C, 53·3; H, 6·2; N, 4·8%.)

m-*Derivatives.* Three rabbits receiving 1 g. each of *m*-cresol yielded 5 g. of glucuronide gum. Half of this gum on methylation and acetylation yielded 1 g. of *triacetyl β-m-cresylglucuronide methyl ester*, m.p. 93–94° (from ligroin) and $[\alpha]_D^{21°}$ −25·6° (*c* = 1 in $CHCl_3$). (Found: C, 56·7; H, 5·8; OMe, 7·4%.) The mother liquors from the acetyl ester treated with NH_3 yielded *β-m-cresylglucuronidamide*, m.p. 222–224° (from ethanol-ether) and $[\alpha]_D^{20°}$ −68·7° (*c* = 0·4 in ethanol). (Found: C, 52·7; H, 6·2; N, 4·8%.)

p-*Derivatives.* Five rabbits each receiving 0·5 g. *p*-cresol yielded 4·36 g. of crude glucuronide gum, 3·5 g. of which yielded 0·73 g. of *triacetyl β-p-cresylglucuronide methyl ester*, m.p. 140° and $[\alpha]_D^{20°}$ −36° (*c* = 1 in $CHCl_3$). (Found: C, 56·7; H, 5·8; OMe, 7·3%.) *β-p-Cresylglucuronidamide* formed white needles, m.p. 257–259° from methanol-water with $[\alpha]_D^{23°}$ −76·6° (*c* = 0·1 in methanol). (Found: C, 52·2; H, 6·1; N, 4·7. $C_{13}H_{17}O_6N$, H_2O requires C, 51·8; H, 6·4; N, 4·7%.)

Phenylphenylglucuronides (diphenylylglucuronides)

o-*Derivatives.* Four rabbits were each fed with 2·5 g. of the sodium salt of *o*-hydroxydiphenyl (British Drug Houses Ltd.) and a 6 hr. urine collected. The urine was acidified to congo red and extracted continuously with ether for 16 hr. The extract was evaporated to a syrup which was dissolved in water, and freed from unchanged *o*-hydroxydiphenyl by shaking with light petroleum. The residue was evaporated to dryness and then methylated in methanol with ethereal diazomethane. Evaporation of the solvents yielded the neutral methyl ester as a gum. (This crystallized on one occasion but was not analysed and it showed $[\alpha]_D^{20°}$ −63° in ethanol and m.p. 123° not sharp.) The ester was acetylated with 4 ml. acetic anhydride and 1 drop of 60% perchloric acid, and after 0·5 hr. the mixture was diluted with water to yield 0·5 g. of crystalline *triacetyl β-o-phenylphenylglucuronide methyl ester*, m.p. 132° and $[\alpha]_D^{20°}$ −85·3° with no mutarotation (*c* = 1 in $CHCl_3$). (Found: C, 61·4; H, 5·5. $C_{25}H_{26}O_{10}$ requires C, 61·7; H, 5·4%.) On treatment with methanolic ammonia for 14 hr. the compound yielded *triacetyl β-o-phenylphenylglucuronidamide* in small yield. This formed colourless plates, m.p. 192–196° and $[\alpha]_D^{25°}$ −68° (*c* = 1 in ethanol). (Found: C, 60·7; H, 5·6; N, 2·8. $C_{24}H_{25}O_9N$ requires C, 61·1; H, 5·4; N, 3·0%.)

p-*Derivatives.* *p*-Phenylphenylglucuronide (m.p. 183° and $[\alpha]_D^{25°}$ −85·3°, 1% in ethanol) was prepared biosynthetically according to Dodgson, Garton, Stubbs & Williams (1948). On methylation with diazomethane it yielded the crystalline *methyl ester*, m.p. 192–193° and $[\alpha]_D^{20°}$ −81·8° (*c* = 1 in ethanol). (Found: C, 61·4; H, 5·4. $C_{19}H_{20}O_7$, 0·5 H_2O requires C, 61·8; H, 5·7%.) Acetylation of this ester (0·75 g.) yielded *triacetyl β-p-phenylphenylglucuronide methyl ester* (0·85 g.) m.p. 170–171° (from aqueous methanol) and $[\alpha]_D^{20°}$ −30·3° (*c* = 1 in $CHCl_3$). (Found: C, 61·4; H, 5·3%.) On deacetylation with methanolic ammonia, p-*phenylphenylglucuronidamide* was obtained as a very insoluble white crystalline powder, m.p. 277° (decomp.). It was too sparingly soluble to permit measurement of its rotation. (Found: C, 62·8; H, 5·6. $C_{18}H_{19}O_6N$ requires C, 62·6; H, 5·5%.) Treatment of this amide with acetic anhydride and a trace of perchloric acid followed by dilution of the acetylation mixture with water yielded *triacetyl* p-*phenylphenylglucuronidamide* as colourless needles (from ethanol), m.p. 207–210° and $[\alpha]_D^{20°}$ −14° (*c* = 2 in $CHCl_3$). (Found: C, 60·8; H, 5·2. $C_{24}H_{25}O_9N$ requires C, 61·1; H, 5·3%.)

Derivatives of the glucuronides of catechol, resorcinol and quinol (with B. Spencer)

β-o-Methoxyphenylglucuronidamide. This *compound* was prepared by the action of ammonia on a methanolic solution of triacetyl *o*-methoxyphenylglucuronide methyl ester (Garton & Williams, 1948). It crystallized from the reaction mixture as colourless needles, m.p. 216–217° (after recrystallization from 90% ethanol) and $[\alpha]_D^{22°}$ −84° (*c* = 0·2 in methanol). (Found: C, 50·1; H, 5·8. $C_{13}H_{17}O_7N$, 0·5 H_2O requires C, 50·6; H, 5·9%.) Acetylation of this compound (0·1 g.) with pyridine and acetic anhydride gave *triacetyl β-o-methoxyphenylglucuronidamide* (0·102 g.) as colourless needles (from aqueous ethanol), m.p. 144° and $[\alpha]_D^{22°}$ −61·4° (*c* = 0·2 in $CHCl_3$). (Found: C, 53·2; H, 5·2. $C_{19}H_{23}O_{10}N$ requires C, 53·6; H, 5·4%.)

β-m-Hydroxyphenylglucuronidamide. Deacetylation of triacetyl *m*-acetoxyphenylglucuronide methyl ester (1 g.) (Garton & Williams, 1949) with methanolic ammonia yielded *β-m-hydroxyphenylglucuronidamide* (0·512 g.) as colourless needles (from ethanol-ether), m.p. 213° and $[\alpha]_D^{22°}$ −81·6° (*c* = 0·25 in methanol). (Found: C, 49·0; H, 5·5; N, 5·2. $C_{12}H_{15}O_7N$, 0·5 H_2O requires C, 49·2; H, 5·7; N, 4·8%.) On methylation, this compound (0·3 g.) yielded *β-m-methoxyphenylglucuronidamide* (0·305 g.), m.p. 210–211° and $[\alpha]_D^{22°}$ −75·5° (*c* = 0·25 in methanol). (Found: C, 50·9; H, 5·7.) The last compound (0·1 g.) on acetylation yielded *triacetyl β-m-methoxyphenylglucuronidamide* (0·107 mg.) as colourless needles (aqueous ethanol), m.p. 127° and $[\alpha]_D^{22°}$ −12·9° (*c* = 0·3 in ethanol) and −26·3° (*c* = 0·3 in $CHCl_3$). (Found: C, 53·4; H, 5·3%.)

β-p-Hydroxyphenylglucuronidamide. In a similar manner, *β-p-hydroxyphenylglucuronidamide*, m.p. 227–230° and $[\alpha]_D^{22°}$ −85·7° (*c* = 0·25 in methanol) (Found: C, 48·5; H, 5·4; N, 5·0), *β-p-methoxyphenylglucuronidamide*, m.p. 238–240° and $[\alpha]_D^{25°}$ −81·7° (*c* = 0·14 in methanol) (Found: C, 51·4; H, 5·7. $C_{13}H_{17}O_7N$ requires C, 52·0; H, 5·7), and *triacetyl β-p-methoxyphenylglucuronidamide*, m.p. 143–144° and $[\alpha]_D^{24°}$ −28·7° (*c* = 0·25 in $CHCl_3$) (Found: C, 53·5; H, 5·6%) were prepared from triacetyl 4-acetoxyphenylglucuronide methyl ester (Garton & Williams, 1949).

DISCUSSION

The molecular rotations in chloroform of the triacetyl methyl esters of some substituted β-phenyl-D-glucuronides are given in Table 1, from which it is clear that the molecular rotations, $[M]_D$, of the *para*-substituted derivatives (approx. −13000 to

Table 1. *Molecular rotations of the triacetyl methyl esters of substituted β-phenyl-D-glucuronides*

($[M]_D$ measured in 1% solution in $CHCl_3$; temperature 20° in most cases and up to 25° in others. Values for atomic and group radii and for interatomic distance from Wheland (1949) and Remick (1949).)

Substituent group	$[M]_D/100$ *ortho*	*meta*	*para*	$\Delta[M]_D/100$ *o–p*	Atomic or group radius (A.)	Inter-atomic distance (A.)	References to rotations
H	−135	−135	−135	0	0·94	1·08	Parke & Williams (1951)
F	−192	—	−142	−50	1·39	1·34	This paper
Cl	−288	—	−146	−142	1·89	1·69	This paper
Br	−316	−162	−136	−180	2·11	1·88	This paper
I	−339	—	−138	−201	2·20	2·00	This paper
CH_3	−199	−107	−152	−47	1·73	—	This paper
C_6H_5	−415	—	−147	−268	—	—	This paper
CN	−309	−161	−183	−126	—	—	Smith (1949)
NO_2	+84	−194	−232	+316	1·92	—	Robinson *et al.* (1951)

−15,000), except *p*-NO_2 and *p*-CN, are not very different from that of the unsubstituted triacetyl phenylglucuronide methyl ester (−13,500). The molecular rotations for the *ortho*-substituted derivatives, however, are much more negative than those of the *para*-isomers. This suggests that the *o*-substituents may be involved in steric hindrance with the acetylated sugar ring and so restrict the free rotation of the phenyl ring about the bonds joining it with the glycosidic oxygen. Examination of molecular models shows that steric hindrance might be expected between the *ortho* substituent and the acetoxy group on C-2 of the glucuronic acid; there is apparently little or no hindrance between the *ortho*-substituent and a hydroxyl group on C-2. Figs. 1 and 2 are drawings made from models of *o*-chlorophenylglucuronide and its triacetyl derivative, using the ball and spoke models described by Wooster, McGowan & Moore (1949) and made by Crystal Structures Ltd., Cambridge. The pyranose ring of the glucuronide is shown in that chair conformation which makes all the substituents equatorial, since Reeves (1950) has shown that this is the stable conformation for the hexopyranosides.

Examination of Stuart models of the type described by Settatree, Thomas & Yardley (1950) and made by Catalin Ltd., Waltham Abbey, Essex, confirmed these views on steric hindrance.

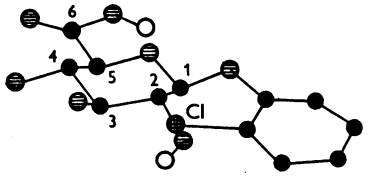

Fig. 1. Drawing made from a ball and spoke model of β-*o*-chlorophenylglucuronide. H atoms on the benzene and carbohydrate rings have been omitted for the sake of clarity. ● = C; ⊖ = O; ⊕ = Cl; ○ = H (see text).

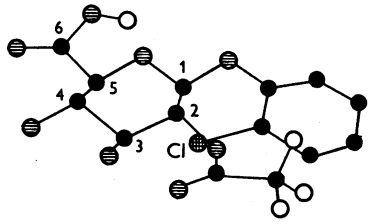

Fig. 2. Drawing of triacetyl β-*o*-chlorophenylglucuronide. Acetyl groups on C-3 and C-4 of the glucuronide have been omitted; other atoms omitted as in Fig. 1.

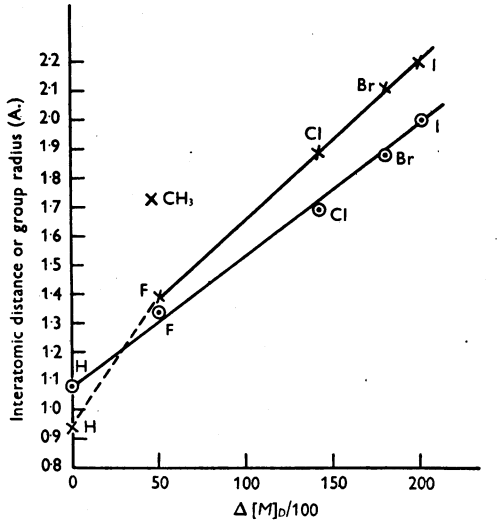

Fig. 3. The relation between the differences in molecular rotation, $\Delta[M]_D/100$ between the *ortho*- and *para*-derivatives of the triacetyl methyl esters of halogen substituted β-phenyl-D-glucuronides, and the halogen-carbon interatomic distances (⊙—⊙) or the atomic (or group) radii (×—×).

Some support for the view that steric hindrance is involved has been obtained with the halogen substituted derivatives by plotting the carbon-halogen interatomic distances (taken from Wheland, 1944) against the differences in molecular rotation

Table 2. *Molecular rotations of substituted β-phenyl-D-glucuronidamides in methanol*

Substituent group	$[M]_D/100$ *ortho*	*meta*	*para*	*para*-glucuronide*	References
H	−188	−188	−188	−244	Parke & Williams (1951)
F	−204	—	−221	—	Garton, Robinson & Williams (1949)
Cl	−217	−257	−260	−250	Spencer & Williams (1950)
Br	−225†	−209†	−212†	−238	This paper
CH_3	−201‡	−194‡	−217†	−215	This paper and Bray, Thorpe & White (1950)
OH	—	−240	−252	—	This paper
OCH_3	−251	−233	−244	—	This paper
CN	−236†	−197	−218†	−288	Smith (1949)
NO_2	−200‡	−294	−319	—	Robinson *et al.* (1951)

* The molecular rotation in water of the free *p*-glucuronide is included here for comparison.
† In water.
‡ In ethanol.

($\Delta[M]_D$) between the *ortho*- and *para*-derivatives (see Fig. 3). A straight-line relationship is obtained showing that $\Delta[M]_D$ is proportional to the size of the *o*-substituent. A similar result is obtained when atomic radii (Wheland, 1949) are plotted against $\Delta[M]_D$. In this case we can include the CH_3 group for which a group radius has been quoted. It is to be noted, however, that it does not fall in line with the halogens for its $\Delta[M]_D$ is much less than expected if the relationship given in Fig. 3 is true. This may be because the CH_3 group can be distorted. Table 2 gives the molecular rotations of some non-acetylated phenylglucuronidamides and glucuronides and shows that the molecular rotations of the *o*-, *m*- and *p*-derivatives are roughly the same, suggesting that the steric effect noted above only occurs when the glucuronide is acetylated. The effect, however, shows up again in the triacetyl amides, although here we can only quote three examples (Table 3).

Table 3. *Molecular rotations of some triacetyl β-phenyl-D-glucuronidamides*

(Solvent, $CHCl_3$—except for chloro compounds, which are in ethanol.)

Substituent group	$[M]_D/100$ *ortho*	*meta*	*para*	Reference
H	−130	−130	−130	Parke & Williams (1951)
Cl	−194	− 57	− 46	Spencer & Williams (1950)
OCH_3	−261	−112	−122	This paper
C_6H_5	−320	—	− 66	This paper

The diphenyl derivatives

Much of our knowledge of the phenomenon of restricted rotation has been obtained from the study of substituted diphenyl derivatives (for reviews see Wheland, 1949; Barnett, 1950). We therefore prepared the triacetyl methyl esters of the glucuronides of *o*- and *p*-hydroxydiphenyl and found that whereas the *p*-derivative shows a normal molecular rotation (−14,700) the *o*-derivative possesses an abnormally high negative rotation (−41,500) which is nearly three times the $[M]_D$ of the *para*-isomer. Here we could have not only restricted rotation of the bonds *a* and *b* between the carbohydrate ring and its attached phenyl group but also restricted rotation between the two phenyl rings at bond *c*. These possibilities can be visualized from Figs. 4 and 5. However, an examination of the spectra of

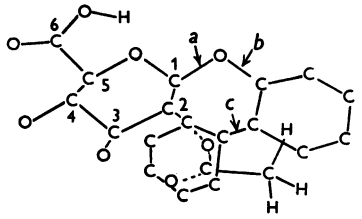

Fig. 4. Drawing of triacetyl β-*o*-phenylphenylglucuronic acid. Atoms and groups omitted as in Fig. 2.

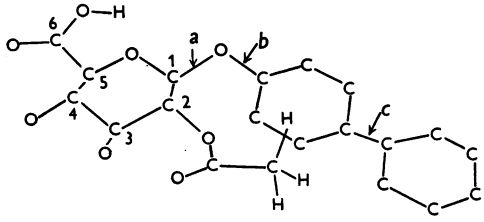

Fig. 5. Drawing of triacetyl β-*p*-phenylphenylglucuronic acid. Atoms and groups omitted as in Fig. 2.

the hydroxydiphenyls and the triacetyl methyl esters of their glucuronides (see Table 4) suggests that in the diphenyl system of both acetylated glucuronides the two benzene rings are coplanar. The molecular extinctions ($\epsilon_{max.}$) of the main bands of these glucuronides (*o*-, 12,600 and *p*-, 20,200) are of the same order as that of diphenyl itself (20,000), the high extinction being due to pronounced conjugation between the two aromatic rings (Braude, 1945) which is only possible if they are coplanar. If

Table 4. *Absorption spectra of the hydroxydiphenyls and their glucuronides*

(Solvent, ethanol—except for benzene and diphenyl which were measured in hexane.)

Compound	$\lambda_{max.}$ (mμ.)	$\epsilon_{max.}$	Reference
Benzene	256	250	Braude (1945)
Diphenylmethane	261	502	Magat & Maier (1943)
Diphenyl	246	20,000	Braude (1945)
p-Hydroxydiphenyl	260	17,600	This paper
Triacetyl methyl ester of *β*-*p*-glucuronosidodiphenyl	252–8	20,200	This paper
o-Hydroxydiphenyl	246 (290)*	10,000 (4,700)	This paper
Triacetyl methyl ester of *β*-*o*-glucuronosidodiphenyl	245 (278)*	12,600 (2,600)	This paper

* Secondary band.

they were not coplanar, resonance between the two rings would be prevented and the molecular extinctions might be expected to be lower, perhaps about 500 (as in diphenylmethane) which is the sum of the extinctions of the 256 mμ. band of two separate benzene rings. Wheland (1944) quotes among other examples dimesityl, whose absorption spectrum is almost identical (per aromatic ring) with that of mesitylene but very different from that of diphenyl itself (Pickett, Walter & France, 1936; O'Shaughnessy & Rodebush, 1940). In dimesityl planar arrangement of the two rings is made impossible by the steric interactions of methyl groups. The abnormal optical rotation of triacetyl *β*-*o*-phenylphenylglucuronide methyl ester would therefore appear to be due to hindered rotation about the glycosidic linkage. The glucuronide showed no mutarotation.

SUMMARY

1. A number of phenols have been fed to rabbits and the *β*-glucuronides have been isolated from the urine. The glucuronides have been converted to amides and triacetyl methyl esters. The phenols used were *o*- and *p*-fluoro-, *o*- and *p*-chloro-, *o*-, *m*- and *p*-bromo- and *o*- and *p*-iodophenols; *o*-, *m*- and *p*-cresols; *o*- and *p*-hydroxydiphenyls. Seventeen new triacetyl methyl esters are described.

2. The molecular rotations of these compounds have been determined and the triacetyl methyl esters of most of the *o*-substituted phenylglucuronides show higher negative rotations than the corresponding *m*- and *p*-isomers. This effect is not shown by the non-acetylated glucuronidamides.

3. In the case of the triacetyl methyl esters of the *o*-halogenophenylglucuronides, it appears that the molecular rotation is directly related to the atomic radius of the substituent or to the length of the carbon-halogen link. The abnormality may be due to restricted rotation about the bond joining the phenyl group to the glycosidic oxygen atom.

4. The abnormally high negative rotation of the triacetyl methyl ester of *β*-*o*-phenylphenylglucuronide as compared with its *p*-isomer suggests that the phenomenon of restricted rotation may be involved here also. Spectroscopic data suggest that there is no restriction of rotation between the two phenyl rings of this compound.

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REFERENCES

Barnett, E. B. (1950). *Stereochemistry*, p. 56. London: Pitman.

Bray, H. G., Thorpe, W. V. & White, K. (1950). *Biochem. J.* **46**, 275.

Braude, E. A. (1945). *Ann. Rep. Chem. Soc.* **42**, 124.

Dodgson, K. S., Garton, G. A., Stubbs, A. L. & Williams, R. T. (1948). *Biochem. J.* **42**, 357.

Garton, G. A., Robinson, D. & Williams, R. T. (1949). *Biochem. J.* **45**, 65.

Garton, G. A. & Williams, R. T. (1948). *Biochem. J.* **43**, 206.

Garton, G. A. & Williams, R. T. (1949). *Biochem. J.* **44**, 234.

Magat, M. & Maier, N. (1943). *Spectres d'Absorption des Liquides, solutions et solides*, vol. 13 (Années 1931–1936), Section 33, p. 46. Paris: Hermann et Cie.

O'Shaughnessy, M. T. & Rodebush, W. H. (1940). *J. Amer. chem. Soc.* **62**, 2906.

Parke, D. V. & Williams, R. T. (1951). *Biochem. J.* **48**, 621.

Pickett, L. W., Walter, G. F. & France, H. (1936). *J. Amer. chem. Soc.* **58**, 2296.

Reeves, R. E. (1950). *J. Amer. chem. Soc.* **72**, 1499.

Remick, A. E. (1949). *Electronic Interpretations of Organic Chemistry*, 2nd ed. p. 294. New York: Wiley.

Robinson, D., Smith, J. N. & Williams, R. T. (1951). *Biochem. J.* **50**, 221.

Settatree, A. A., Thomas, S. L. & Yardley, V. A. (1950). *Nature, Lond.*, **166**, 59.

Smith, J. N. (1949). *Biochem. J.* **45**, 638.

Spencer, B. & Williams, R. T. (1950). *Biochem. J.* **47**, 279.

Wheland, G. W. (1944). *The Theory of Resonance* (especially pp. 160, 292). New York: Wiley.

Wheland, G. W. (1949). *Advanced Organic Chemistry*, 2nd ed. p. 210. New York: Wiley.

Wooster, N., McGowan, J. C. & Moore, W. T. (1949). *J. sci. Instrum.* **26**, 140.