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Synthesis of 4-*O*-(β-D-Glucopyranosiduronic Acid)-dopamine

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4-*O*-(β-D-Glucopyranosiduronic acid)-dopamine was synthesized for comparison with a substance of identical properties present in a golden hamster islet cell tumour. The compound was prepared by ion pair alkylation of tetrabutylammonium 2-benzyloxy-4-formylphenolate with methyl (2,3,4-tri-*O*-acetyl-α-D-glucopyranosyl bromide) uronate followed by nitromethylation of the aldehyde function, catalytic hydrogenation of the nitrostyrene derivative to a saturated amine with simultaneous removal of the benzylic group and a final hydrolysis of the ester functions. The ion pair alkylation afforded a very convenient route to this glycoside.

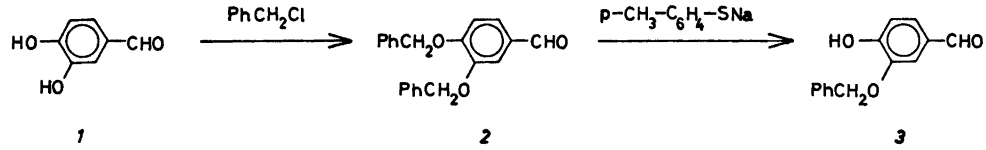
In studies on catechol and indole derivatives in a transplantable islet cell tumour in the golden hamster we have obtained chromatographic and spectroscopic evidence suggesting the presence of an unusual biogenic amine. This substance on treatment with formaldehyde and glyoxylic acid vapour gives a strongly fluorescent derivative with emission at much shorter wavelength than is usual for similar derivatives from biogenic catecholamines. Biochemical and chemical data [1] indicate that the substance is a glucuronide of dopamine with the glycosidic linkage in the 4-position. Monoglucuronides of catecholamines have been found earlier [2] but the position of the conjugation has never been well established and they have never been prepared synthetically before. To obtain more definite proof of the structure and to obtain material for histochemical and pharmacological studies we decided to make a total synthesis of the most probable compound, 4-*O*-(β-D-glucopyranosiduronic acid)-dopamine. The synthetic material showed identical fluorescence spectrum and R_F-value to the compound obtained from the golden hamster islet cell tumour.

RESULTS AND DISCUSSION

The desired product, *8*, has two main components, the sugar and the aglycon. The sugar component was protected as the fully acetylated methyl uronate and this was converted to methyl (2,3,4-tri-*O*-acetyl-α-D-glucopyranosyl bromide) uronate *4* according to known procedures.[3] In the aglycon part we circumvented the need for nitrogen protection by insertion of the nitrogen part of the molecule after formation of the glycosidic linkage and we protected the 3-oxygen atom with a benzylic group which could easily be removed by catalytic hydrogenation under very mild conditions which would leave the glycosidic bond intact. As a point of attack for insertion of the nitrogen-containing fragment of the molecule we decided to use the easily available aldehyde group.

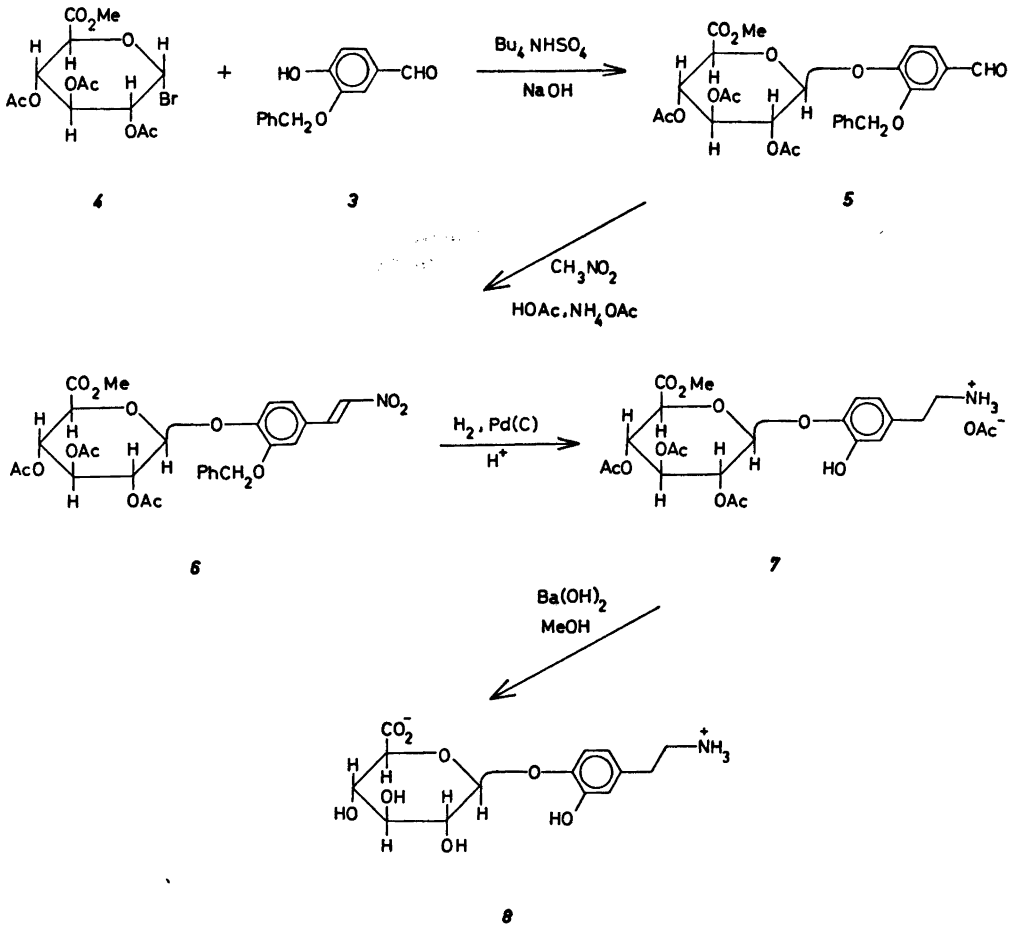
Selective protection of the 3-oxygen of the 3,4-dihydroxybenzaldehyde (*1*) by benzylation in strong alkali [4] was tedious with a difficult isomer separation and furthermore gave a low yield. These problems were, however, avoided by full benzylation to 3,4-dibenzyloxybenzaldehyde *2* and selective debenzylation to 3-benzyloxy-4-hydroxybenzaldehyde *3*. It was carried out by nucleophilic substitution using the very strong nucleophile sodium *p*-thiocresolate to attack the more electrophilic benzyl group in the 4-position [5] (Scheme 1).

The formation of the glycosidic bond presented serious difficulties as most of the usual glycosidation reactions failed entirely or gave



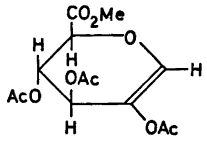
Scheme 1.

very low yields. Thus, coupling of 3-benzyloxy-4-hydroxybenzaldehyde, *3*, with methyl (tri-*O*-acetyl-α-D-glucopyranosyl bromide) uronate, *4*, using lithium hydroxide in methanol [6] gave a difficultly separable mixture which contained only about 10 % of the desired product *5*. Cadmium carbonate in toluene has been successfully used as base for a similar glycosidation of steroid phenols [7] but gave in this case about the same yield. However, by performing the glycosidic coupling by ion pair alkylation [8] we succeeded in obtaining the desired product, *5*, in 28 % yield (Scheme 2). To our knowledge this is the first time ion pair alkylation has been used in the synthesis of a glycosidic bond. Tetrabutylammonium 2-benzyloxy-4-formylphenolate was extracted with methylene chloride from an aqueous solution of tetrabutyl-



Scheme 2.

ammonium hydrogen sulfate and excess sodium hydroxide and reacted with methyl (tri-*O*-acetyl-α-D-glucopyranosyl bromide) uronate, *4*. The principal byproduct was methyl 2,3,4-tri-*O*-acetyl-1-deoxy-D-arabinohex-1-ene uronate, *9*, (37 %) formed by internal elimination of hydrogen bromide. This elimination product has been reported as the main product on attempted formation of glycosides of certain complex phenols.[9] The starting aldehyde *3* was used in slight excess and 50 % was recovered.



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As the yield of the tetrabutylammonium phenolate might have been lowered by formation of an adduct of free phenol and the tetrabutylammonium phenolate [10] we also tried the use of an equimolar 50 % sodium hydroxide solution with a one-step extraction and alkylation, but this method was inferior to the two-step procedure. It should be noted that this is a rather special case with a very lipophilic phenol and a formyl group in the *para* position.

The ion pair alkylation procedure gave a β-glycosidic linkage since the product, *5*, was identical with products obtained by reactions known to give the β-anomer, for example by use of lithium hydroxide in methanol. The β-linkage was also corroborated by the optical rotations. These comply with Hudson's rule of isorotation for *5* as well as for the subsequent derivatives. Further the IR spectrum showed absorption at 890 cm^{-1}, characteristic for β-anomers, but no absorption at 845 cm^{-1}, characteristic for α-anomers.[11]

The glycosidic aldehyde *5* was reacted with nitromethane in absolute ethanol with acetic acid/ammonium acetate as a catalyst [12] giving the nitrostyrene derivative *6* (76 %). The nitrostyrene *6* was then hydrogenated catalytically in acetic acid with a small amount of concentrated sulfuric acid [13] and a palladium catalyst. The reaction was very fast even at atmospheric pressure and the benzyl group was removed in the same reaction. To minimize acidic cleavage of the glycoside, the mineral acid was removed by passing the reaction mixture through an anion exchanger in acetate form.

Hydrolysis of the dopamine acetate glucuronide *7* with aqueous barium hydroxide in methanol gave the desired 4-*O*-(β-D-glucopyranosyluronic acid)-dopamine, *8*, in 94 % yield. This dopamine glucuronide was compared with material from the hamster transplantable islet cell tumour. Both showed the same R_F-value on paper chromatography and their fluorescent derivatives from reaction with glyoxylic acid showed the same characteristic fluorescence spectrum. The synthetic product, *8*, was cleaved by β-glucoronidase giving free dopamine in the same way as the biological material. The β-configuration was further established by IR data and optical rotation, as for the glycosidic aldehyde *5*. The NMR spectrum of *8* gave a coupling constant of 6.6 Hz between the H-1′ and H-2′ protons and thus according to the Karplus curve must correspond to the β-glycosidic product. The combined evidence confirms the structure of the synthetic product and indicates its equivalence with the substance found in the biological preparation.

EXPERIMENTAL

All melting points are uncorrected. IR spectra (in KBr) were measured with a Perkin-Elmer Modell 257 spectrophotometer. NMR spectra were recorded on a Jeol JNM-MH-100 NMR-spectrometer or a Varian T-60 spectrometer. TLC was performed using Merck Fertigplatten F_{254}, silica gel, dimethyl sulfoxide impregnated silica gel, and cellulose. For visualization of the compounds, UV light, silver nitrate (2 % aq) spray followed by sodium hydroxide (2 M) and 10 % sulfuric acid spray followed by heating at 120 °C for 15 min were used. The following solvent systems were used; A (chloroform – ethyl acetate, 3:1), B (ethyl acetate-light petroleum, 2:1), C (ethyl ether saturated with dimethyl sulfoxide), D (phenol – 0.1 M hydrochloric acid, 1:9). Elemental analyses were performed by Centrala Analyslaboratoriet, Uppsala.

*Methyl(2,3,4-tri-*O*-acetyl-α-D-glucopyranosyl bromide) uronate* (*4*) was prepared accordingly to Ref. 3.

3-Benzyloxy-4-hydroxybenzaldehyde (*3*). 3,4-Dibenzyloxybenzaldehyde,[14] *2*, (1.58 g) and a slight excess of sodium *p*-thiocresolate (950 mg) were refluxed under nitrogen in sodium-dried toluene (15 ml) with a small amount of HMPA

(1.15 ml) for 20 h. The reaction was monitored on TLC using silica gel plates and solvent system A. When 3,4-dibenzyloxybenzaldehyde had disappeared, methylene chloride (35 ml) was added and 3-benzyloxy-4-hydroxybenzaldehyde was extracted with ten portions of 2 M sodium hydroxide. The aqueous phase was collected and acidified with concentrated HCl to pH 1 and the 3-benzyloxy-4-hydroxybenzaldehyde was reextracted with methylene chloride. The methylene chloride phase was washed with water and dried over sodium sulfate. Concentration under reduced pressure on a Rotavapor gave pure (TLC, NMR) crystals which could be used without further purification or recrystallised from ethanol. Yield 1.10 g (96 %). M.p. 112–113 °C, lit.[4] 114 °C. ^{1}H NMR (60 MHz, $CDCl_3$): δ 5.13 (s, 2 H), 7.04 (d, 1 H, J 8.0 Hz), 7.40 (m, 7 H), 9.80 (s, 1 H). IR: (KBr) 3500–3000, 1660, 1590, 1507 cm^{-1}.

*Methyl(2-benzyloxy-4-formylphenyl 2',3',4'-tri-*O*-acetyl-β-*D*-glucopyranoside) uronate* (*5*). 3-Benzyloxy-4-hydroxybenzaldehyde, *3*, (3.2 g) was dissolved in methylene chloride (50 ml). Solutions of sodium hydroxide (5.6 g) in water (15 ml) and tetrabutylammonium hydrogen sulfate (7.0 g) in water (25 ml) were prepared. The three solutions were mixed and shaken vigorously for 10 min. The methylene chloride phase was carefully separated and diluted with ethyl ether (50 ml). Methyl(tri-*O*-acetylglucopyranosyl bromide) uronate, *4*, (4.0 g) dissolved in methylene chloride–ether 1:1 (100 ml) was added with stirring (1 h at 0 °C). The reaction mixture was left overnight and then concentrated under reduced pressure to 10 ml on a Rotavapor. The residue was chromatographed on silica gel (deactivated with 2.5 % water) using methylene chloride as eluent. The eluate was monitored by a UV-detector. As soon as the starting aldehyde *3* had been eluted, 5 % ether was added to the eluent thus to give the desired product, *5*, in a rather small volume. The product fraction was evaporated to dryness giving a gelatinous residue (2.8 g), which was crystallised by dissolving in a small volume of methylene chloride, adding ether until opalescence appeared and then leaving in a refrigator overnight; this gave white crystals (0.9 g) of pure (TLC, NMR) product *5*. From the mother liquor a second crop of pure product (0.6 g) was isolated, yield 1.5 g (28 %). Recrystallisation from methylene chloride-ether gave m.p. 148.5–149.5 °C. $[\alpha]_D^{25}$ −83.7° (*c* 1, chloroform). ^{1}H NMR (60 MHz, $CDCl_3$): δ 1.80 (s, 3 H), 2.04 (s, 6 H), 3.72 (s, 3 H), 4.05–4.30 (m, 1 H), 5.15 (s, 2 H), 5.35 (m, 4 H), 7.40 (m, 8 H), 9.85 (s, 1 H), IR: (KBr) 1770, 1705, 1600, 1514, 1235, 1090, 1045, 895 cm^{-1}. Anal. $C_{27}H_{28}O_{12}$: C, H.

Evaporation of the mother liquor and recrystallisation of the solid residue from ethanol gave methyl 2,3,4-tri-*O*-acetyl-1-deoxy-D-arabinohex-1-ene uronate *9* (1.2 g, 37 %), m.p. 74–76 °C, lit.[9] 76 °C.

The first fraction of the eluate gave 1.65 g (50 %) recovery of the starting aldehyde *3*.

*Methyl(2-benzyloxy-4-(2-nitrovinyl)phenyl 2',3',4'-tri-*O*-acetyl-β-*D*-glucopyranoside) uronate* (*6*). Methyl(2-benzyloxy-4-formylphenyl-tri-*O*-acetyl-β-D-glucopyranoside) uronate *5* (850 mg) was dissolved in absolute ethanol (25 ml). Ammonium acetate (0.5 g), acetic acid (0.5 ml), and a few drops of acetic anhydride and nitromethane (1.1 ml) were added and the mixture was heated with stirring at 60 °C overnight. Methylene chloride (25 ml) was then added and the solution was washed twice with water. After evaporation to dryness the residue was dissolved in a small volume of methylene chloride and then ether was added until opalescence appeared. The mixture was left in a refrigator overnight giving 600 mg of the desired glycosidic nitrostyrene *6* as yellow crystals. Another crop (90 mg) of the product *6* was collected from the mother liquors. Total yield 690 mg (76 %), m.p. 205–210 °C. After two recrystallisations from methylene chloride-ether, the melting point was 208–210 °C. $[\alpha]_D^{25}$ −71.7° (*c* 1, chloroform), ^{1}H NMR (60 MHz, $CDCl_3$): δ 1.80 (s, 3 H), 2.03 (s, 6 H), 3.70 (s, 3 H), 4.05–4.30 (m, 1 H), 5.10 (s, 2 H), 5.30 (m, 4 H), 7.00–8.00 (m, 10 H). IR (KBr): 1770, 1645, 1610, 1599, 1530, 1517, 1235, 1107, 1045, 898 cm^{-1}. Anal. $C_{28}H_{29}NO_{13}$: C, H, O.

*Methyl(4-(2-ammonioethyl)-2-hydroxyphenyl 2',3',4'-tri-*O*-acetyl-β-*D*-glucopyranoside) uronate acetate* (*7*). Methyl(2-benzyloxy-4-(2-nitrovinyl)-phenyl 2',3',4'-tri-*O*-acetyl-β-D-glucopyranoside) uronate *6* (256 mg) was hydrogenated in acetic acid (5 ml), with concentrated sulfuric acid (75 μl) and palladium (5 % on carbon, 100 mg) which had been previously saturated with hydrogen. With vigorous stirring at atmospheric pressure and room temperature the calculated amount of hydrogen was absorbed within 1 h. After removal of the catalyst, excess acetic acid was evaporated under reduced pressure with continuous addition of ethanol (70 %) to maintain the volume at about 5 ml. The residue was passed through an anion exchange resin, Amberlite CG 4B in acetate form, and eluted with ethanol 75 %. The eluate was evaporated under reduced pressure and the residual syrup was crystallized by adding ether. The white crystals formed were collected and washed with ether giving 165 mg (70 %) of the desired product *7*, m.p. 125–127 °C. $[\alpha]_D^{25}$ −27.1° (*c* 0.2, methanol). ^{1}H NMR (100 MHz, CD_3OD): δ 1.90 (s, 3 H), 2.05 (s, 9 H), 2.84 (t, 2 H, J 6.6 Hz), 3.12 (t, 2 H, J 6.6 Hz), 3.70 (s, 3 H), 4.39 (d, 1 H, J 9.6 Hz), 5.05–5.55 (m, 4 H), 6.62 (m, 2 H), 6.95 (d, 1 H, J 8.1 Hz). IR (KBr): 3700–3200, 1765, 1580, 1515, 1235, 1105, 1050, 895 cm^{-1}. Anal. $C_{23}H_{31}NO_{13}$: C, H. N.

*4-*O*-(β-*D*-glucopyranosiduronic acid)-dopamine* (*8*). The glycosylated dopamine acetate *7* (132 mg) was treated with aqueous barium

hydroxide (5 ml 0.15 M) and methanol (5 ml) at 60 °C for 3 h. Acetic acid (1 ml) was then added and the barium was precipitated by adding concentrated sulfuric acid (100 μl). After centrifugation the supernatant was passed through an anion exchange resin, Amberlite CG-400 in acetate form, and the product was eluted with water. The eluate on evaporation under reduced pressure at 30 °C gave a dried residue of white amorphous material which could not be induced to crystallize, yield 78.7 mg (94 %). $[\alpha]_D^{25}$ −54.0° (*c* 0.8, water). ^{1}H NMR (100 MHz, D_2O): δ 2.83 (t, 2 H, *J* 6.6 Hz), 3.19 (t, 2 H, *J* 6.6 Hz), 3.67 (m, 3 H), 4.15 (d, 1 H, *J* 9.0 Hz), 4.73 (broad s), 5.05 (d, 1 H, *J* 6.6 Hz), 6.76 (m, 2 H), 7.05 (d, 1 H, *J* 8.1 Hz). IR (KBr): 3700 – 3000, 1565, 1415, 875 cm^{-1}. Found: C 50.09; H 6.04; N 3.97. Calc. for $C_{14}H_{19}NO_8$: C 51.06; H 5.82; N 4.25.

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REFERENCES

1. Falck, B., Hansson, C., Kennedy, B.-M. and Rosengren, E. *Acta Physiol. Scand. In press.*
2. Dodgson, K. S. and Williams, R. T. *Biochem. J. 45* (1949) 381; Clark, W. G., Akawie, R. I., Pogrund, R. S. and Geissman, T. A. *J. Pharmacol. Exp. Therap. 101* (1951) 6; Hertting, G. *Biochem. Pharmacol. 13* (1964) 1119.
3. Bollenbeack, G. N., Long, J. W., Benjamin, D. G. and Lindquist, J. A. *J. Am. Chem. Soc. 77* (1955) 3310.
4. Funke, A. and Paulsen, A. *Gazz. Chim. Ital. 91* (1961) 1268.
5. Hansson, C. and Wickberg, B. *Synthesis* (1976) 191.
6. Berrang, B., Twinw, C. E., Henessee, G. L. and Carrol, F. I. *Synth. Commun. 5* (1975) 231.
7. Conrow, R. B. and Bernstein, S. *J. Org. Chem. 36* (1971) 863.
8. Brändström, A. and Ljunggren, U. *Acta Chem. Scand. 23* (1969) 2204.
9. Andersson, F. B. and Leaback, D. H. *Chem. Ind. London* (1960) 967.
10. Modin, R. and Tilly, A. *Acta Pharm. Suec. 5* (1968) 311.
11. Nakanishi, K. *Infrared Absorption Spectroscopy*, Holden-Day, San Francisco and Mankodo Co., Tokyo 1969, p. 34.
12. Rao, M. G. S., Srikantia, C. and Iyengar, M. S. *Helv. Chim. Acta 12* (1929) 581.
13. Kindler, K., Brandt, E. and Gehlhaar, E. *Justus Liebigs Ann. Chem. 511* (1934) 209.
14. Bristow, N. W. *J. Chem. Soc.* (1957) 513.

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