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Note

O- and *C*-D-glucosyluronic acid derivatives of Δ^1-tetrahydrocannabinol: Synthesis and differential behavior to β-glucuronidase*†

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Cannabinoids form D-glucosyluronic acid derivatives as *in vivo* metabolites[1,2], as well as on incubation with UDP–D-glucopyranosyluronic acid transferase in the presence of uridinediphospho-D-glucopyranosyluronic acid[3,4]. Earlier work[3,5] from this laboratory examined the possibility that these D-glucosyluronic acid derivatives are of both the *O*- and *C*-types. For purposes of comparison, the *O*- and *C*-D-glucosyluronic acid derivatives of Δ^6-tetrahydrocannabinol[6] were synthesized[3,6], and the synthetic products were instrumental in demonstrating that the uncommon *C*-D-glucosyluronic acids were indeed formed both in the *in vitro*[3] and in the *in vivo* reactions[5]. In our synthesis, strongly acidic conditions were employed[5].

Currently, our interest in synthesizing the Δ^1-tetrahydrocannabinol derivatives resides in the observation that Δ^1-tetrahydrocannabinol is the *main* active constituent of Cannabis[7,8] and is used as an antiemetic drug in cancer chemotherapy. As Δ^1-tetrahydrocannabinol isomerizes to Δ^6-tetrahydrocannabinol, we avoided an acid-catalyzed condensation step in the synthesis of the D-glucosyluronic acid derivative.

Methyl (2,3,4-tri-*O*-acetyl-β-D-glucopyranosyl bromide)uronate[9] was condensed with Δ^6-tetrahydrocannabinol in the presence of mercuric cyanide (*cf.* ref. 10), to yield the known[3] compounds **1** and **2**. The same procedure was then applied to Δ^1-tetrahydrocannabinol and yielded compounds **3** and **5**. Compound **3** appeared to be (by t.l.c. and h.p.l.c.) the pure β-D anomer, showing a low optical rotation and a doublet at δ 4.80 (*J* 10 Hz) that was assigned to the anomeric proton, in analogy[3] to the anomeric proton (δ 4.90, *J* 8 Hz) of **1**. Compound **5** (δ 4.67, ~*J* 8 Hz) is probably also the β-D anomer, based on analogy[3] to **2**, but in this case, this could not be supported further by enzymic evidence. The chemical shift of the free aromatic

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†The nomenclature used in this paper is based on the monoterpenoid numbering. The formal (I.U.P.A.C.) numbering is given in parentheses in structure **1** (*Editor*).
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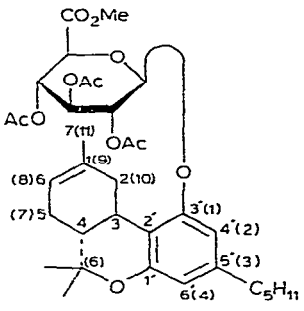

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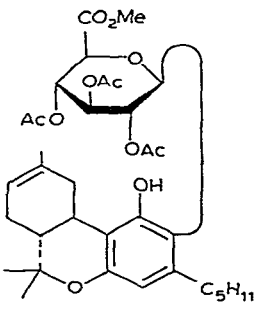

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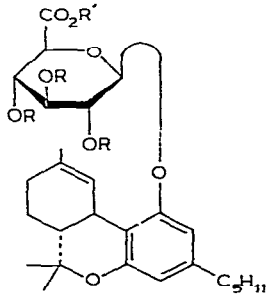
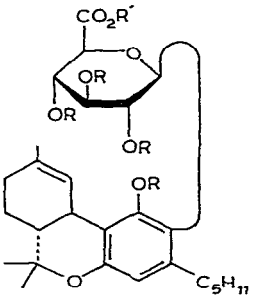


3 R = Ac, R′ = Me
4 R = H, R′ = H



5 R = Ac, R′ = Me
6 R = H, R′ = H

proton of **5** (δ 6.67) is identical to that of **2** (ref. 3), and is different from that of the known Δ^6-tetrahydrocannabinyl D-glucosyluronic acid derivative substituted at C-6′ of the cannabinyl derivative[3] (δ 6.38). This supports the assignment of a C-4′ substitution for **5**. Compounds **3** and **5** were saponified to give **4** and **6**, respectively. As expected, **4**, the *O*-glucosyl derivative, was a substrate for β-D-glucuronidase[11,12], whereas **6**, the *C*-glucosyl derivative, was unaffected by the enzyme. This observation may be of considerable importance in the cannabinoid field, since it is assumed that the major conjugation pathway of cannabinoid metabolism takes place by formation of its D-glucosyluronic acid derivative, and it has been shown that water-soluble "conjugates" of cannabinoids in liver or urine are hydrolyzed to a limited extent only by β-D-glucuronidase[13,14].

The present synthesis may be of interest, beyond the cannabinoid field, since *C*-glucosyluronic acid formation was shown to be a general metabolic pathway[5,15,16], and synthetic methods leading to *C*-glucosyluronic acid derivatives may be needed for investigations in this new area.

EXPERIMENTAL

General methods. — T.l.c. was conducted on aluminum sheets coated with Silica gel 60 F_{254} (Merck), and p.l.c. on Silica gel GF (Merck), both being eluted

with 1:1 (v/v) ether–petroleum ether, unless otherwise stated. Chromatograms were viewed under u.v. light or sprayed with sulfuric acid. Samples were isolated from p.l.c. plates by viewing under u.v. light, scraping off, and extracting the bands with chloroform. All products were oils, and their purity was ascertained by t.l.c. Silica Wolem TSC and active, neutral aluminum oxide (Merck) were used for column chromatography. G.l.c. was performed at 220° in a column (90 cm) filled with 3% of OV on Chromosorb W and eluted at a rate of 50 mL/h. H.p.l.c. was performed on a column (0.6 cm dia. × 25 cm Varian) of CH 10, eluted at a rate of 60 mL/h, and equipped with a u.v. detector set at 230 nm; compounds were isolated from the outlet of the detector. N.m.r. spectra were recorded with a Bruker 60 MHz instrument, and mass spectra with an Atlas CH5 instrument. No satisfactory analysis for compounds **3**–**6** could be obtained.

Δ^1-Tetrahydrocannabinol was isolated from hashish[7], and Δ^6-tetrahydrocannabinol was prepared by boiling cannabidiol with *p*-toluenesulfonic acid.

*Methyl (Δ⁶-tetrahydrocannabinyl 2,3,4-tri-*O*-acetyl-β-*D*-glucopyranosyl)uronate* (**1**) *and 4′-(methyl 2,3,4-tri-*O*-acetyl-β-*D*-glucopyranosyluronate) Δ⁶-tetrahydrocannabinol* (**2**). — Methyl (2,3,4-tri-*O*-acetyl-β-D-glucopyranosyl bromide)uronate (1 g, 2.65 mmol) which was freshly prepared from methyl 1,2,3,4-tetra-*O*-acetyl-α,β-D-glucopyranuronate), Δ^6-tetrahydrocannabinol (110 mg, 0.35 mmol), and mercuric cyanide (740 mg), in a 1:1 (v/v) benzene–nitromethane (10 mL) mixture were kept with stirring under a nitrogen atmosphere for 8 h at 40°, and then for 16 h under reflux. The mixture was filtered through a Celite filter in the hood, and the residue washed with excess dichloromethane. The solution was washed with concentrated sodium chloride, dried (magnesium sulfate), and evaporated, and the products were isolated by p.l.c. Compounds **1** (13 mg, 7%) and **2** (39 mg, 20%) were found to be identical with authentic samples[3] by t.l.c. and m.s. The two compounds were acetylated overnight by treatment with pyridine (0.3 mL) and acetic anhydride (0.2 mL) at room temperature. Ice was added, and the reaction mixture was kept for 1 h at room temperature, and evaporated *in vacuo*. Compound **1** (unchanged) and 3′-*O*-acetyl-4′-(methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate)-Δ^6-tetrahydrocannabinol were found to be identical with authentic samples[3] (t.l.c., m.s.).

*Methyl (Δ¹-tetrahydrocannabinyl 2,3,4-tri-*O*-acetyl-β-*D*-glucopyranosyl)uronate* (**3**) *and 3′-*O*-acetyl-(4′-methyl 2,3,4-tri-*O*-acetyl-β-*D*-glucopyranosyluronate)-Δ¹-tetrahydrocannabinol* (**5**). — Methyl (2,3,4-tri-*O*-acetyl-α-D-glucopyranosyl bromide) uronate, freshly prepared from methyl 1,2,3,4-tetra-*O*-acetyl-α,β-D-glucopyranuronate (3 g, 7.96 mmol), was treated with Δ^1-tetrahydrocannabinol (0.3 g, 0.96 mmol), as described in the preceding paragraph. Following evaporation, g.l.c. demonstrated that at least 90% of the excess Δ^1-tetrahydrocannabinol remained intact and had not isomerized to Δ^6-tetrahydrocannabinol after being subjected to the reaction conditions. Pyridine (20 mL) and acetic anhydride (10 mL) were added to the residue, and the reaction mixture was kept overnight at 0°. Ice was then added and the solution was evaporated *in vacuo*. The mixture obtained was applied to an alumina column (50 g, 2-cm dia.) and 19-mL fractions were collected. After an initial

volume of 190 mL, **3** was eluted with ether (190 mL, 5 mg, 0.8%), followed by a mixture of **3** and **5** (720 mL, 5% ethyl acetate in ether, 56 mg, 5 and 4%, respectively). Compound **5** emerged as the next fraction (340 mL, 10% ethyl acetate in ether, 19 mg, 4%). The products were further separated and purified by p.l.c.

Compound **3**: $[\alpha]_D^{24}$ −86.6° (*c* 1.5, ethanol); ^{1}H-n.m.r. (chloroform-*d*): δ 6.40 (s), 6.20 (s), 5.40 (m), 4.80 (d, w, *J* 10 Hz), 4.20 (d, m, *J* 7 Hz), 3.80 (CO_2CH_3), 3.20 (m), 2.06 ($OCOCH_3$), 2.03 ($OCOCH_3$), 1.73 (OAc-3′), 1.40 [pyrane ring, cannabinoid $(CH_3)_2$], 1.26, 1.06, and 0.93; m.s.: *m/z* 630 (M), 511, 451, 422, 342, and 280.

Compound **5**: $[\alpha]_D^{23}$ −91.8° (*c* 0.3, ethanol); ^{1}H-n.m.r. (chloroform-*d*): δ 6.67 (s), 5.90, 5.31 (m), 4.67 (~d, *J* 8 Hz), 4.06 (~d, *J* 8 Hz), 3.73 (CO_2CH_3), 2.76 (m), 2.40 (phenolic $OCOCH_3$), 2.05 ($OCOCH_3$), 2.00 ($OCOCH_3$), 1.85 (C-3′, $OCOCH_3$), 1.60 [pyran ring, cannabinoid $(CH_3)_2$], 1.28, 1.06, and 1.00; m.s.: *m/z* 672 (M), 653, 630, 613, 570, 553, 509, 493, 467, 451, 421, 391, 367, 355, 342, 270 and 269.

Δ^1-Tetrahydrocannabinyl β-D-glucopyranosiduronic acid (**4**) *and 4′-(β-D-glucopyranosyluronic acid)-Δ^1-tetrahydrocannabinol* (**6**). — A sample (~7 mg) of **3** or **5** was dissolved in acetone (1.8 mL) and M sodium hydroxide solution (0.2 mL) was added. The reaction mixture was kept at room temperature until the value of optical rotation changed little[17] (~20 min.). The solution was passed through Dowex 50 (H^+) cation-exchange resin contained in a Pasteur pipette, which was further washed with 2:1 (v/v) acetone–water. The eluates (pH 5) were partially evaporated under nitrogen, and then lyophilized. T.l.c. (2:3, v/v, acetone–ethyl acetate) showed in each case, a major polar product (**4** and **6**, respectively) close to the origin. H.p.l.c. (20% acetonitrile in water) was used to follow the formation of the products and to isolate them in pure form. Elution times were: for **4**, 144 s (starting material **3**, 288 s); for **6**, 216 s (reference α-naphthol, 921 s.). Free, unglycosylated cannabinoids have the following retention times (in 55% acetonitrile): Δ^1- and Δ^6-tetrahydrocannabinol, 242 s.; cannabinol acetate, 125 s (α-naphthol 101 s).

Compounds **4** and **6** were further characterized by m.s. of the per-*O*-(trimethylsilyl) derivatives. Samples purified by h.p.l.c. were evaporated and dissolved in the silylation reagent (0.1 mL; 2:3:9, v/v, trimethylsilyl chloride–hexamethyldisilazane–pyridine) and the mixture was kept for 20 min at room temperature. The solution was evaporated *in vacuo*, the residue dissolved in a little dichloromethane, and the mass spectrum determined.

M.s. of **4** (at 115°): *m/z* 778 (M), 760, 747, 705 (M − Me_3Si), 687, 581, 571, 523, 511, 500, 481, 439, 428, 415, 399, 385, 327, 313, and 309.

M.s. of **6** (at 150°): *m/z* 848 (M − 2, weak), 818, 777 (M − Me_2Si), 686, 558, 540, 528, and 499.

β-D-Glucuronidase digestion. — A solution of β-D-glucuronidase (beef liver, 12 million Fishman units/g, Sigma) (40 mg/mL) and a saturated solution of either **4** or **6** (1:8 by volume) in 4:1 (v/v) sodium acetate–acetic acid buffer (0.1M, pH 4.45)

and 1,3-propanediol containing α-naphthol (as an internal standard) were incubated for 48 h at 37° under nitrogen. Aliquots (10 mL) were examined by h.p.l.c. (20% acetonitrile in water). The amount of **4** decreased by 69%, whereas that of **6** remained unchanged.

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