

Short Communication

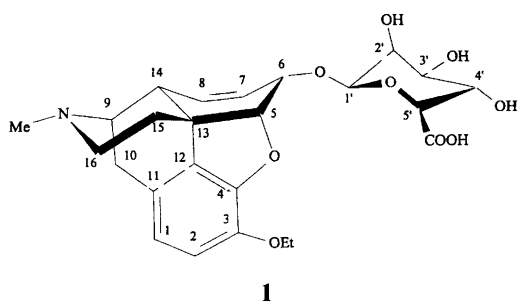
Synthesis of Ethylmorphine-6-glucuronide: a Metabolite of Ethylmorphine in Man

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The drug 3-*O*-ethylmorphine (ethylmorphine) is used as an antitussive, a chemotic in ophthalmology, and as an analgesic in veterinary medicine. 3-*O*-Ethylmorphin-6-yl-β-*D*-glucopyranosiduronic acid (ethylmorphine-6-glucuronide, EM6G1) is assumed to be the major metabolite of ethylmorphine in man.^{1–3} This metabolite has not previously been synthesized and, thus, is not commercially available. The primary aim of the present study was to synthesize EM6G for use as reference material in a study of the biotransformation of ethylmorphine in man.⁴



Synthesis and structure

The synthesis of EM6G was based on the synthesis of the corresponding codeine glucuronide by Yoshimura *et al.*⁵ Oxidation of codeine to codeinone with an excess of silver carbonate in boiling benzene has been described by Rapoport and Reist.⁶ A similar oxidation of ethylmorphine was not observed. The reaction was followed by

TLC. No significant difference in the product mixture was observed on addition of silver carbonate in two large or in several small portions. In both cases one large spot ($R_f = 0.73$), identified as AcEM6G, and two minor spots ($R_f = 0.56$) and ($R_f = 0.92$) were seen on the TLC plate. Separation of the reaction mixture by flash chromatography furnished the AcEM6G as a crystalline product which has hydrolysed by alkali to EM6G.

Alkaline hydrolysis of AcEM6G according to Yoshimura *et al.*⁵ gave the glucuronide EM6G as an amorphous powder. The molecular formula of EM6G ($C_{25}H_{31}NO_9$) was consistent with the molecular ion (m/z 489) in the mass spectrum (Fig. 1).

Nuclear magnetic resonance (NMR) spectroscopy confirmed the identity of EM6G. All signals in the spectrum were assigned using two-dimensional proton–proton correlation spectroscopy (COSY) (Fig. 2). The proton shifts in D_2O were assigned in accordance with those reported by Xu *et al.*⁷ for norethylmorphine in $CDCl_3$ except that the chemical shifts of the protons 7 and 8 were interchanged.

The ^{13}C NMR chemical shifts in D_2O (Table 1) were assigned in accordance with the shifts of ethylmorphine in $CDCl_3$ and morphine-6-glucuronide in D_2O reported by Carrupt *et al.*⁸ and Xu *et al.*,⁷ respectively. The chemical shift (104.7 ppm) of C1 in the glucuronic acid moiety is in agreement with corresponding shifts given for morphine-6-glucuronide (104.39 ppm) and methyl β-*D*-glucopyranosiduronic acid (104.3 ppm) (Gorin *et al.*⁹) suggesting the β-configuration at the anomeric carbon. This is supported by the spin–spin coupling constant (J 7.7 Hz) between H-1 and H-2 of the glucuronic acid moiety.

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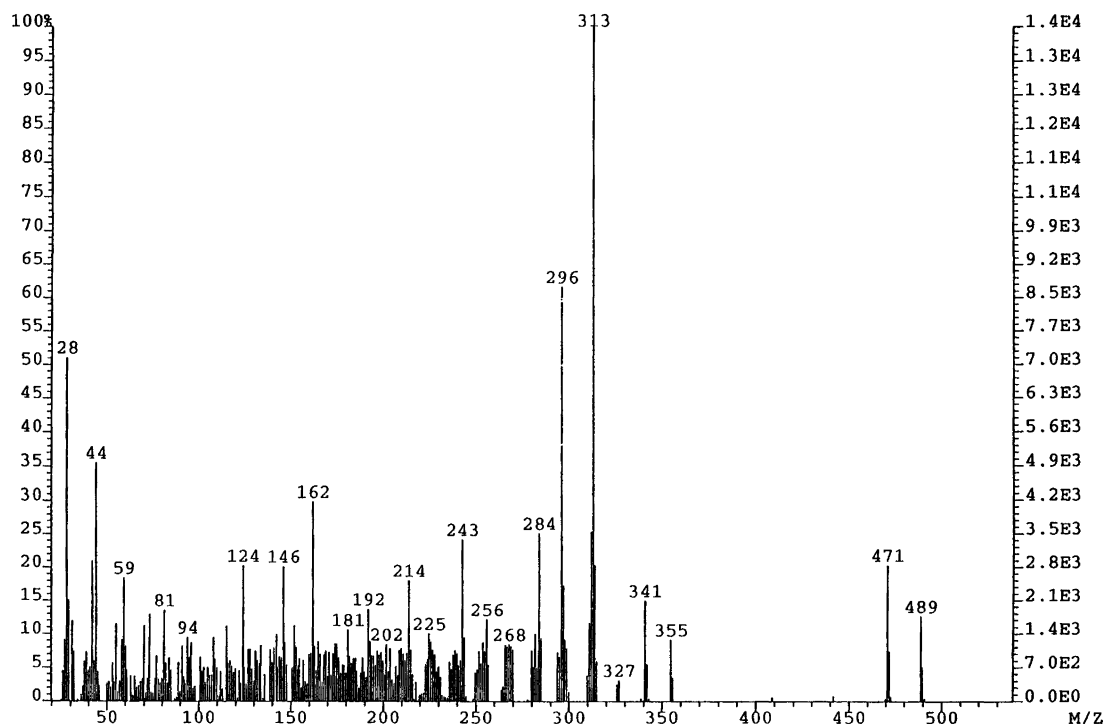


Fig. 1. Mass spectrum of ethylmorphine-6-glucuronide.

Synthesized EM6G was hydrolysed with β -glucuronidase in water and analysed by GC-MS.¹⁰ The only reaction product seen was ethylmorphine, further confirming the structure of EM6G as a β -glucuronide.

Confirmation of EM6G as a metabolite of ethylmorphine

Biotransformation studies of ethylmorphine conjugation with UDP-glucuronic acid to EM6G have so far been hampered by the lack of EM6G as a reference substance. The identification of EM6G as a major metabolite of ethylmorphine has till now been indirect. Biological samples have been subjected to acidic or enzymatic hydrolysis (Gerdin *et al.*,² Rane *et al.*³) followed by determination of the increase in the ethylmorphine concentration. In the present study human urine and blood samples, collected after intake of ethylmorphine, were analysed. The identity of the peak considered to be EM6G in the HPLC chromatograms of urine and blood extracts was confirmed by (a) co-chromatography of blood and urine extracts together with synthesized EM6G and (b) analysis of authentic urine and blank urine spiked with synthesized EM6G before and after hydrolysis with hydrochloric acid. After hydrolysis the HPLC chromatogram showed that the peak considered to be EM6G had disappeared, while GC-MS analysis showed an increase in ethylmorphine concentration corresponding to the loss of EM6G.

Experimental

All chemicals and solvents were reagent or HPLC grade and used as such. Ethylmorphine hydrochloride was obtained from Weiders Farmasøytiske A/S (Norway). Bromo-2,3,4-tri-*O*-acetyl- α -D-glucopyranuronic acid methyl ester (acetobromo- α -D-glucuronic acid methyl ester) and β -glucuronidase (Limpets *Patella vulgata* type L-II) were purchased from Sigma (USA). Other reagents were obtained from the following suppliers: pentafluoropropionic acid anhydride (PFPA), Pierce Europe B.V. (The Netherlands); flash chromatography silica (Silica gel 60, 15–40 μ m), Merck (Germany); SepPack light SPE-cartridges, Waters (USA); Bond Elute C₁₈ and Bond Elute Certify SPE-cartridges, Varian (USA).

'Active' silver carbonate was prepared from anhydrous sodium carbonate and silver nitrate according to Wolfrom and Lineback.¹¹

Ammonium sulfate buffers, 0.5 M and 5 mM, were prepared from ammonium sulfate and adjusted to pH 9.3 with diluted ammonia. Phosphate buffer, 10 mM, was prepared from sodium dihydrogen phosphate and adjusted to pH 2.1 with phosphoric acid.

Methods. NMR spectra were recorded on a Varian Gemini-200 instrument operating at 200 MHz (¹H) and 50 MHz (¹³C); the solvents were CDCl₃ or D₂O. Chemical shifts are reported in ppm relative to tetramethylsilane. Two-dimensional proton-proton correlation spectroscopy (COSY) was carried out for the assignment of the proton signals in the spectrum of EM6G. Mass spec-

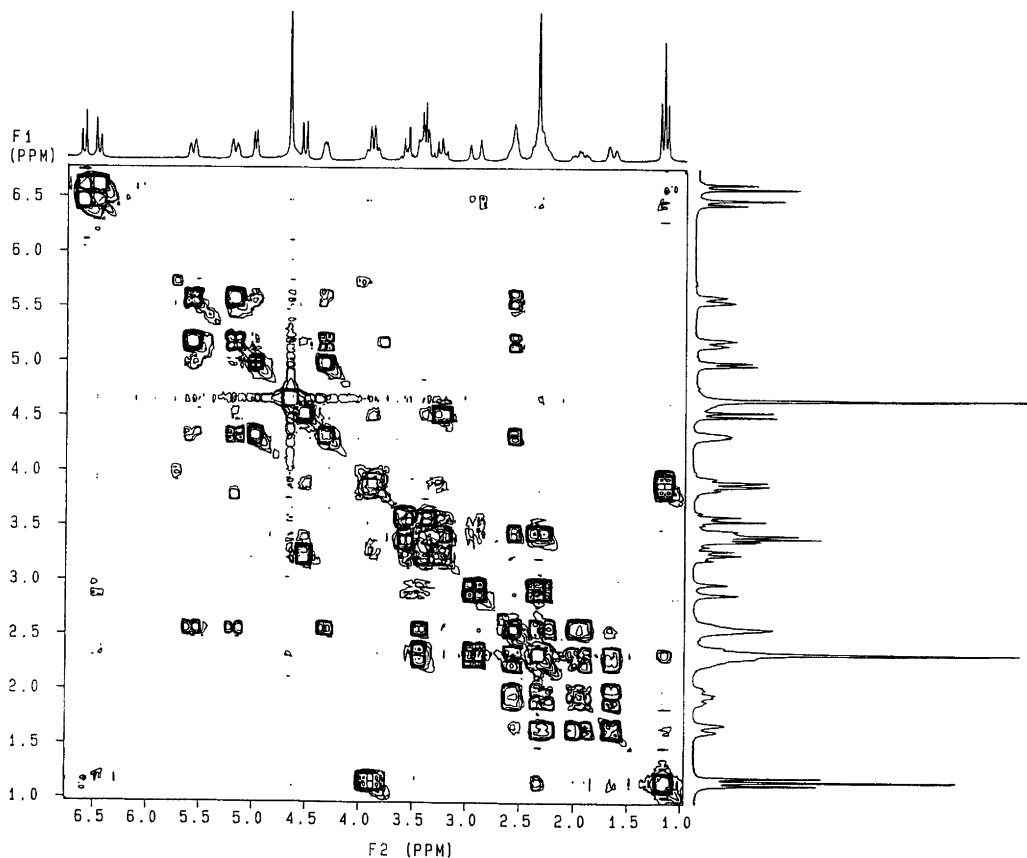


Fig. 2. Two-dimensional proton correlation spectrum (COSY) of ethylmorphine-6-glucuronide. (D_2O as the solvent, shift positions in ppm relative to tetramethylsilane).

tra (direct inlet) were recorded on a VG ProSpec Mass spectrometer, Fisons Instruments (UK). Capillary gas chromatography–mass spectrometry (GC–MS) was performed on a Hewlett-Packard GC–MS system equipped with an HP 5970A mass selective detector, Hewlett-Packard (USA) according to Gjerde *et al.*¹⁰ Thin layer chromatography (TLC) was carried out on silica gel plates, 0.2 mm layer, Whatman International (UK). Solvent systems were (A) chloroform–methanol (4:1), (B) acetonitrile–phosphate buffer pH 2.1 (26:74), (C) ethanol–water (4:1). Detection: Acidified iodine platinate or 10% v/v H_2SO_4 in EtOH, 105°C. R_f -values (solvent system): ethylmorphine; 0.53 (A), 0.49 (B), AcEM6G; 0.73 (A), 0.46 (B), EM6G; 0.69 (B), 0.29 (C) irregular spot.

Methyl [3-O-ethylmorphin-6-yl-2',3',4'-tri-O-acetyl- β -D-glucopyranosyl]uronate (AcEM6G). A benzene–water azeotrope (20 ml) was distilled off from a vigorously stirred and refluxing mixture of ethylmorphine· H_2O (1.65 g; 5.0 mmol) and freshly prepared silver carbonate (3.2 g; 11.2 mmol) in dry benzene (300 ml). To the boiling mixture, a solution of acetobromo- α -D-glucuronic acid methyl ester (4.0 g; 10 mmol) in dry benzene (80 ml) was added dropwise over a period of 7 h. Benzene (about 20 ml) was slowly removed by distillation during the ad-

dition of the acetobromo- α -D-glucuronic acid methyl ester. TLC (system A) revealed remaining ethylmorphine and additional silver carbonate (3.2 g; 11.2 mmol) was added. The reaction mixture was refluxed for 3 h; benzene (50 ml) was slowly distilled off during this period. More benzene (50 ml) was added and the mixture was refluxed overnight. TLC (system A) revealed three products (R_f 0.55; R_f 0.73 and R_f 0.92); ethylmorphine (R_f 0.44) was not detected. The mixture was filtered and concentrated *in vacuo* to about 50 ml. The solution was extracted with 0.5% aqueous hydrochloric acid (3×100 ml). The aqueous phase was made alkaline to pH 8 with sodium hydrogen carbonate and extracted with chloroform (5×100 ml). The combined extracts were dried with sodium sulfate and concentrated *in vacuo* to 2.83 g of an amorphous solid. Flash chromatography (silica gel; chloroform–ethanol 10:1, 5 ml min^{-1}) afforded 1.38 g of a crystalline product. Recrystallisation from ethanol furnished colourless needle-like crystals. Yield 1.09 g (35%), m.p. 158–161°C. *Anal.* calc. for $C_{32}H_{39}NO_{12}$: C, H, N. $[\alpha]_D^{22} -169.4^\circ$ (c 5.7, $CHCl_3$). 1H NMR (200 MHz, $CDCl_3$): δ 1.35 (t, $J = 7.0$ Hz), 1.87–1.93 (d[†], $J = 11.0$ Hz), 2.02 (s), 2.03 (s), 2.15 (s), 2.37–2.48 (dd, $J = ca. 4.8, 17.9$), 2.53 (s), 2.70–2.79 (dd,

[†] Doublet with additional minor splittings.

Table 1. ^{13}C and ^1H NMR chemical shift data of ethylmorphine-6-glucuronide.^a

Position	^{13}C (ppm in D_2O) ^{b,d}	^1H (ppm in D_2O) ^c	
1	122.8	6.47	(d, J 8.2)
2	117.8	6.61	(d, J 8.2)
3	143.2		
4	149.4		
5	92.3	4.99	(d, J 6.0)
6	74.8 (1)	4.33	(m)
7	133.2 (2)	5.18	(d, ^e J 9.9)
8	133.1 (2)	5.58	(d, ^e J 9.6)
9	61.5	ca. 3.4	(m)
10	23.9	2.93	(d, J 19.5)
10		2.2–2.4	(m)
11	130.0 (2)		
12	131.4 (2)		
13	44.4 (3)		
14	42.1 (3)	2.5–2.65	(m)
15	36.7	1.66	(d, ^e J ca. 12)
15		1.97	(dt, J ca. 4.9?, 12.1)
16	48.9	2.5–2.65	(m)
16		2.2–2.4	(m)
N-Me	45.6 (3)	2.34	(s)
O- CH_2CH_3	68.6	3.90	(q, J 7.1)
O- CH_2CH_3		3.89	(q, J 7.1)
O- CH_2CH_3	17.3	1.17	(t, J 7.0)
1'	104.7	4.53	(d, J 7.7)
2'	76.6 (1)	3.2–3.35	(m)
3'	79.2	3.35–3.50	(m)
4'	76.1 (1)	3.35–3.50	(m) 4?
5'	78.3	3.55–3.65	(m) 5?
6'	178.3		

^a Multiplicities and spin-spin coupling constants in Hz in the proton spectrum are given in parentheses. ^b Assignments are based on a comparison with those of ethylmorphine⁷ and methyl (methyl β -D-glucopyranoside)uronate.⁹ ^c The chemical shifts were, except for H7 and H8, assigned in accordance with those reported by Xu *et al.*⁷ for norethylmorphine. The shifts given for H7 and H8 by Xu *et al.*⁷ should be interchanged. ^d (1), (2), (3) Assignments may be reversed. ^e Doublet with additional minor splittings. ' Glucuronic acid moiety.

J ?), 2.85 (m), 3.05 (d, J = 19.0), 3.46–3.49 (m), 3.75 (s), 3.99–4.15 (m), 4.90–4.96 (m), 5.04–5.12 (m), 5.20–5.37 (m), 5.72 (d[†]). ^{13}C NMR (CDCl_3): δ 14.9, 20.5, 20.6, 20.8, 35.1, 40.3, 42.7, 43.2, 46.5, 52.9, 59.1, 64.8, 69.4, 71.1, 72.0, 72.5, 72.6, 88.0, 98.3, 115.0, 119.0, 125.8, 127.9, 128.0, 130.1, 130.6, 141.4, 147.6, 167.3, 169.4, 169.8, 170.1. MS [IP 70 eV; m/z (% rel. int.)]: 629 (52, M), 569 (6, [M - 60]), 312 (33, [M - 317]), 296 (100, [M - 333]), 257 (12, [M - 372]), 243 (14, [M - 386]), 197 (14, [M - 432]), 155 (53, [M - 474]), 146 (20, [M - 483]), 127 (29, [M - 502]).

3-O-Ethylmorphin-6-yl- β -D-glucopyranosiduronic acid (EM6G). A 1% methanolic solution of MeONa (8 ml) was added to a stirred suspension of AcEM6G (0.70 g; 1.11 mmol) in 8 ml of dry methanol at room temperature. After 3 h TLC (system A) showed no traces of AcEM6G (R_f 0.73). TLC (system B) revealed two products; (R_f 0.69 and R_f 0.25). The slightly turbid solution was stirred overnight and the solvent evaporated off *in vacuo*. Aqueous barium hydroxide (5 ml, 0.25 M) was added to the residue and the mixture stirred for 1 h at room tempera-

ture, heated for 5 min at 100°C, diluted with water (15 ml) and filtered. The residue on the filter was treated with water at 100°C, cooled and filtered. The pH of the combined filtrates was adjusted to 6 with aqueous oxalic acid (0.25 M), filtered and the filtrate evaporated *in vacuo* leaving 0.56 g of an amorphous solid. The reaction product was dissolved in water. On addition of methanol 0.05 g of non-melting white crystals precipitated. The mother liquor gave after evaporation *in vacuo* 0.42 g of an amorphous solid. 0.23 g of this solid was subjected to flash chromatography (silica gel, 12 g; ethanol–water (4:1); 2.5 ml min⁻¹). The fractions which contained one product only, R_f 0.29, TLC (system C), were concentrated *in vacuo* to 0.11 g (30%) of an amorphous solid. Anal. $\text{C}_{25}\text{H}_{31}\text{NO}_9 \cdot 1\frac{1}{2} \text{H}_2\text{O}$: C, H, N (hygroscopic), $[\alpha]_{\text{D}}^{22} - 193.1^\circ$ (c 5.4 mg/ml⁻¹ H_2O). MS spectrum (IP 70 eV) is shown in Fig. 1. ^1H NMR and ^{13}C NMR spectral data are given in Table 1.

Acidic and enzymatic hydrolysis. Aliquots of drug-free urine spiked with authentic EM6G were hydrolysed by being heated with an equal volume of 6 M HCl at 80°C for 15 h according to Rane *et al.*³ Authentic EM6G in water was also incubated with 30000 U ml⁻¹ gluco-

[†] Doublet with additional minor splittings.

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ronidase at 37°C for 48 h. The samples were extracted on Bond Elut Certify™ columns, Varian (USA) by the method recommended by the manufacturer for opiate extractions. The eluates were evaporated to dryness, derivatized (PFP-derivate) with pentafluoropropionic acid anhydride and analysed by GC/MS.¹⁰ EM6G in serum and urine was extracted using an ASPEC robot (Gilson, France) and analysed by high performance liquid chromatography according to Aasmundstad.⁴

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