SYNTHESIS OF 5β-ANDROSTAN-17β-(1'-OXOCYCLOHEX-2'-EN-3'-YL)-3β,14β-DIOL-3-β-D-GLUCOPYRANOSIDE, A POTENT CARDIAC GLUCOSIDE WITH HIGH SAFETY MARGIN

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Abstract - Starting from testosterone 1, an efficient preparation of a cardioactive glucoside 13 with a modified 17β- functionality is described. The glucoside 13, in spite of no lactone group at the 17β-position, is found to be potent and showed a high margin of safety.

The synthesis of digitalis cardenolides has been a difficult and inefficient process due to the high lability and thermodynamically unfavourable geometry of its structural elements. A rapid preparation of many derivatives in sufficient quantities for pharmacological testing is necessary to further design drugs with a margin of safety superior to those of natural cardioactive glycosides. A few years ago an efficient conversion of testosterone to α,β-unsaturated ketone 2 was reported by Wiesner et al[1]. Based on reports published earlier[2] that there might be two separate receptors in the heart muscle for the toxicity and the inotropic activity of natural digitalis glycosides and the lactone ring at 17-position is not essential[3] for the inotropic action, we synthesized and tested the pharmacological properties of a series of steroid glucosides[4] with modified 17β- functionality by extensive use of the intermediate 2. Several modifications of the 17β-lactone ring have also been reported by others[5]. In this paper, we report the synthesis of the glucoside 13 which, in spite of no lactone ring at the 17β-position, showed in pharmacological tests both a potency and a high margin of safety superior to those of natural cardioactive steroid glycosides.

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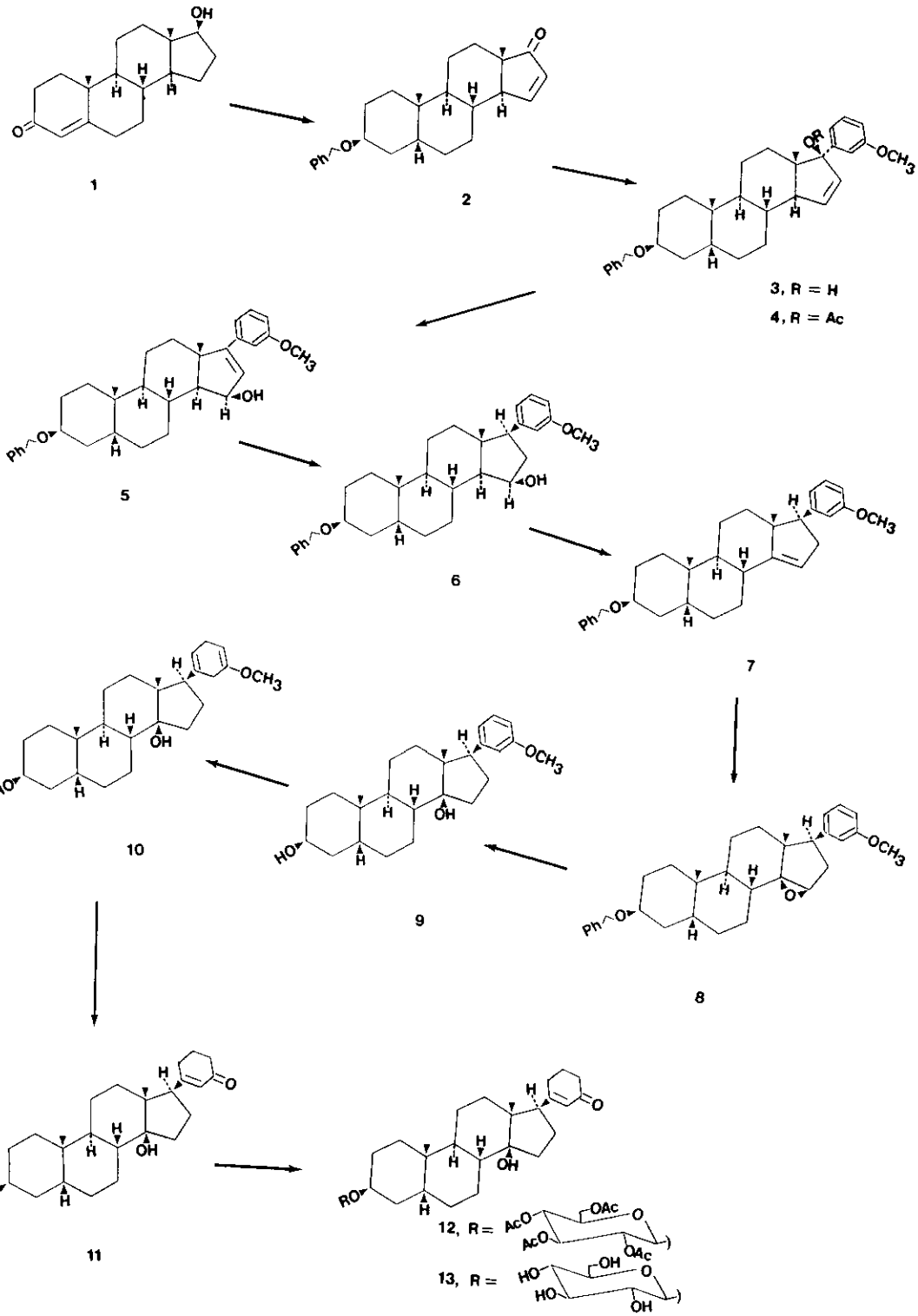
1
2
3, R = H
4, R = Ac
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13, R =

Commercially available testosterone was converted to α,β-unsaturated ketone 2 as described earlier[1], except that L-Selectride[6] was used for the stereoselective reduction of the 3-carbonyl group (THF, 0° C) into the 3β-alcohol (96:4, β:α). The m-anisyllithium was introduced[7] into the enone 2 by a very fast electron transfer reaction in which a mixture of m-bromoanisole and the enone was added into an etheral solution containing lithium slices. The carbinol 3 was acetylated to get 17β-acetate 4 in quantitative yield which without further purification, was subjected to an allylic rearrangement by refluxing it with calcium carbonate in aqueous acetone to yield the alcohol 5 in 70% yield. The allylic alcohol 5 was hydrogenated in ethanol in the presence of KOH over 10% Pd on $CaCO_3$ to yield the saturated alcohol 6 quantitatively. The 15β-hydroxyl group was eliminated from the saturated alcohol 6 by heating it with mesyl chloride in pyridine. The olefin 7 was stirred with NBS, H_2O, and AcOH at room temperature to yield trans-bromohydrin which was further stirred with basic alumina[8] to give the epoxide 8 in 70% yield. The epoxide 8 was refluxed with $LiAlH_4$ in THF to yield the 14β-hydroxyl compound which on hydrogenolysis under normal condition gave 9. The compound 9 was subjected to Birch reduction[9] to yield the dihydro compound 10. In the nmr spectrum, the compound 10 showed 4' vinyl proton as a triplet at $\delta = 4.66$ ($J = 2$ Hz), and 2' proton as a broad singlet at $\delta = 5.63$ ppm. The dihydro compound 10 was stirred with 10% oxalic acid in methanol to give the β,γ-unsaturated compound (4' H at $\delta = 5.76$ ppm) which was further isomerized into the most stable α,β-unsaturated compound 11 in 10% oxalic acid in 65% yield. The compound 11 displayed the 2' vinyl proton as a singlet at $\delta = 5.96$ ppm, and it has an extinction coefficient of 8781.5 in ethanol at A_{245} ($\epsilon = A/Cl$). The compound 11 on glycosylation[10] with silver oxide, $MgSO_4$, and aceto-α-bromo-D-glucose in 1,2-dichloroethane gave acetate 12 which on hydrolysis in ammonia yielded cardenolide analog 13 in 75% yield.

EXPERIMENTAL

Ir spectra were determined in CCl_4 or, in sodium chloride cells with a Perkin-Elmer spectrophotometer, model 237B or model 727B. Nmr spectra were measured using a T-60 and CFT-20 spectrometer in $CDCl_3$ or DMSO-d_6. Uv spectra were recorded on Beckmann 25, and mass spectra were recorded on a Hitachi-Perkin-Elmer RMU-6D. Elemental analyses were performed at Ayerst Research Laboratories, Montreal. All melting points were determined on a Koffler hot stage apparatus, and were uncorrected.

5β-Androst-15-en-17α-m-anisyl-3β,17β-diol 3-benzyl ether 3

To a 500 ml round bottom flask, fitted with a stirrer and a dropping funnel, tetrahydrofuran (200 ml) and lithium slices (600 mg) were introduced under a slow stream of nitrogen and the temperature was reduced to 0° C. The carbonyl compound 2 (15 g) was mixed with an excess of m-bromoanisole (10 ml) and the mixture was transferred to a dropping funnel attached to the reaction vessel and the solution was stirred for 1 h. After 1 h when the dull matt appearance of the lithium surface was changed into golden colour, the addition of the reagents was continued over 2 h. After the reaction was complete, excess of the lithium was filtered off and the tetrahydrofuran was removed in vacuo. The residue was hydrolysed with 5% hydrochloric acid and extracted with ether. The filtrate was dried over anhydrous magnesium sulphate and evaporated to dryness. The residue was chromatographed using ether-hexane (1:4) to furnish 11.6 g of the compound 3 (60%, mp 158-159° C). Ir ($CHCl_3$): 3450 cm^{-1} (hydroxyl). Nmr ($CDCl_3$): δ 7.29 (s, 5H, benzyl aromatic), 6.93 (m, 4H, anisyl protons), 6.18 (d, J=6 Hz, 1H, 16-H), 5.69 (dd, J=6 Hz and 2 Hz, 1H, 15-H), 4.43 (s, 2H, benzylic), 3.83 (s, 3H, methoxyl), 3.63 (broad s, 1H, 3α-H), 1.09 (s, 3H, 19-CH_3), 1.00 (s, 3H, 18-CH_3). Anal. calcd for $C_{33}H_{42}O_3$: C, 81.48; H, 8.64; O, 9.87. Found: C, 81.40; H, 8.64; O, 9.86. Ms: m/z 486.

5β-Androst-16-en-17-m-anisyl-3β,15β-diol 3-benzyl ether 5

Compound 3 (4.46 g) was acetylated with acetic anhydride (5 ml) in pyridine (10 ml) in the presence of a catalytic amount of 4-dimethylaminopyridine (11 mg) at room temperature for 4 days. The reaction mixture was evaporated at 50° C in vacuo and the residue was dissolved in ether, washed with 5% citric acid, 5% sodium bicarbonate, dried over anhydrous magnesium sulphate, and evaporated to dryness in vacuo to give 4 quantitatively. The acetate 4 was used for the next step without further purification. Ir ($CHCl_3$): 1730 cm^{-1} (acetoxy carbonyl). Nmr ($CDCl_3$): δ 7.29 (s, 5H, benzyl aromatic), 6.81 (m, 4H, anisyl), 6.36 (q, J = 6 Hz, 2H, 15-H & 16-H), 4.43 (s, 2H, benzylic), 3.79 (s, 3H, methoxyl), 3.63 (broad s, 1H, 3α-H), 2.03 (s, 3H, acetoxyl), 1.00 (s, 3H, 19-CH_3), 0.96 (s, 3H, 18-CH_3). The acetate 4 (5 g) was refluxed in aqueous acetone (200 ml, 25% water) in the presence of calcium carbonate (2 g) for 7 days. The filtrate of the reaction mixture was evaporated under reduced pressure to remove most of the acetone and the crude product was dissolved in ether. The organic layer was washed with 5% sodium bicarbonate, dried over anhydrous magnesium sulphate and evaporated to dryness.

The product was purified by column chromatography on silica gel to yield the pure allylic alcohol 5 as foam (3.15 g, 70%) after recycling the recovered starting material over five times. Ir ($CHCl_3$): 3620 cm^{-1} (hydroxyl). Nmr ($CDCl_3$): δ 7.30 (s, 5H, benzyl aromatic), 7.04 (m, 4H, anisyl), 6.04 (d, J = 4 Hz, 1H, 16-H), 4.56 (m, 1H, 15α-H), 4.49 (s, 2H, benzylic), 3.79 (s, 3H, methoxyl), 3.73 (broad s, 1H, 3-H), 1.36 (s, 3H, 18-CH_3), 1.06 (s, 3H, 19-CH_3). Anal. calcd dor $C_{33}H_{42}O_3$: C, 81.48; H, 8.64; O, 9.87. Found: C, 81.43; H, 8.62; O, 9.79. Ms: m/z 486.

5β-Androstan-17β-m-anisyl-3β,15β-diol 3-benzyl ether 6.

The allylic alcohol 5 (3.57 g) was made alkaline with 1N methanolic potassium hydroxide (pH 9.5) and hydrogenated in ethanol (50 ml) over 10% palladium/calcium carbonate (714 mg) at room temperature. The catalyst was removed by filtration through celite and the filtrate was evaporated in vacuo to yield the product 6 as an oil (3.3 g, 92%). Ir ($CHCl_3$): 3620 cm^{-1} (hydroxyl). Nmr ($CDCl_3$): δ 7.29 (s, 5H, benzyl aromatic), 6.76 (m, 4H, anisyl), 4.46 (s, 2H, benzylic), 4.39 (broad m, 1H, 3α-H), 0.96 (s, 3H, 19-CH_3), 0.73 (s, 3H, 18-CH_3).

5β-Androst-14-en-17β-m-anisyl-3β-ol benyl ether 7

Compound 6 (185.6 mg) in pyridine (2 ml) was stirred with mesyl chloride (55 mg) at 60° C for 4 h followed by evaporation in vacuo to dryness. The residue was dissolved in ether, and the organic layer was washed with 5% citric acid, 5% sodium bicarbonate, dried over anhydrous magnesium sulphate, and evaporated to dryness. The crude material was purified by preparative thin layer chromatography (ether:hexane; 1:4) to yield the compound 7 (152 mg, 85%) which was crystallized from ether-hexane, mp 116 - 118° C. Nmr ($CDCl_3$): δ 7.29 (s, 5H, aromatic), 7.16 (broad s, 1H, anisyl), 6.79 (t, J = 4 Hz, 3H, anisyl), 5.26 (broad s, 1H, 15-H, vinylic), 4.46 (s, 2H, benzylic), 3.76 (s, 3H, methoxyl), 3.69 (broad s, 1H, 3α-H), 0.93 (s, 3H, 19-CH_3), 0.61 (s, 3H, 18-CH_3). Anal. calcd for $C_{33}H_{42}O_2$: C, 84.25; H, 8.93; O, 6.80. Found: C, 84.25; H, 8.89; O, 6.80. Ms: m/z 470.

5β-Androstan-14β,15β-epoxy-17β-m-anisyl-3β-ol benzyl ether 8

A mixture of the compound 7 (200 mg), acetic acid (0.036 ml) and water (0.6 ml) in acetone (6 ml) was stirred with N-bromoacetamide (58 mg) at room temperature for 1 h. The reaction mixture was diluted with methylene chloride, washed with 5% sodium bisulfite, dried over anhydrous magnesium sulphate, and evaporated at room temperature in vacuo to dryness. The residue was redissolved in acetone (10 ml) and stirred at room temperature with basic alumina (10 times by weight) for 30 min. Alumina was filtered off and the filtrate was evaporated to dryness. The

crude product was purified on preparative silica gel plate (ether:hexane; 1:4) to yield the pure epoxide 8 (115 mg, 55.3%, mp 119-121° C). Nmr ($CDCl_3$): δ 7.28 (s, 5H, benzyl aromatic), 7.73-6.69 (m. 4H, anisyl), 4.46 (s, 2H, benzylic), 3.76 (s, 3H, methoxyl), 3.69 (broad s, 1H, 3α-H), 3.49 (s, 1H, 15α-H), 0.96 (s, 3H, 19-CH_3), 0.63 (s, 3H, 18-CH_3).

5β-Androstan-17β-m-anisyl-3β,14β-diol 9

The epoxide 8 (115 mg) was added to 15 ml of dry tetrahydrofuran containing lithium aluminium hydride (100 mg) and the mixture was refluxed under nitrogen over 1 h. The reaction mixture was cooled down to room temperature and the inorganic salt was filtered off through a celite pad. The filtrate was evaporated to dryness and the residue was taken up in ether and was washed with water, brine, dried over anhydrous magnesium sulphate, and evaporated to dryness. Purification of the residue on the preparative silica gel plate (ether:hexane; 1:2) furnished with 14-β alcohol (113 mg, 95%) as an oil. Ir ($CHCl_3$): 3600, 3450 cm^{-1}(hydroxyl) Nmr ($CDCl_3$): δ 7.28 (s, 5H, benzyl aromatic), 7.28 - 6.69 (m, 4H, anisyl), 4.56 (s, 2H, benzylic), 3.83 (s, 3H, methoxyl), 3.73 (broad s, 1H, 3α-H), 0.96 (s, 3H, 19-CH_3), 0.63 (s, 3H, 18-CH_3). The above alcohol (113 mg) was hydrogenolyzed with 10% palladium on charcoal (23 mg) at an atmospheric pressure, in ethanol (8 ml), for 2 h. The catalyst was filtered off through a celite pad and the filtrate was evaporated to dryness and the residue was purified on a preparative silica gel plate (ether:hexane; 1:1) to yield the compound 9 (80 mg, 86.4%) as foam. Ir ($CHCl_3$): 3600, 3450 cm^{-1} (hydroxyl). Nmr ($CDCl_3$): δ 7.23 - 6.69 (m, 4H, anisyl protons), 4.03 (broad s, 1H, 3α-H), 3.69 (s, 3H, methoxyl), 0.83 (s, 3H, 19-CH_3), 0.49 (s, 3H, 18-CH_3). Anal. calcd for $C_{26}H_{38}O_3$: C, 78.39; H, 9.54; O, 12.06. Found: C, 78.31; H, 9.49; O, 12.01. Ms: m/z 398.

5β-Androstan-17β-(1'-methoxycyclohexa-1',4'-dien-5'-yl)-3-β-D-glucopyranoside 10

To a 200 ml three-necked flask containing ammonia (50 ml), the compound 9 (300 mg) in anhydrous ether (10 ml) was added slowly from a dropping funnel under nitrogen. The mixture was stirred for 10 min and to the resulting solution lithium metal (54 mg, 10 equivalents) was added in small pieces during a 15 min period. The mixture was stirred for 10 min and absolute ethanol (3 ml) was added dropwise over a 10 min period. After the disappearance of the blue color (1 h), the ammonia was evaporated off and the residue was extracted with ether. The ether extract was washed with distilled water, brine, dried over anhydrous magnesium sulphate, and evaporated to dryness to give the crude dihydro derivative 10 (220 mg, 72.3%, mp

95-97°C). Ir ($CHCl_3$): 3640, 3480 cm^{-1} (hydroxyl). Nmr ($CDCl_3$): δ 5.63 (broad s, 1H, 2'H), 4.66 (t, J = 2 Hz, 1H, 4'H), 4.19 (broad s, 1H, 3α-H), 3.59 (s, 3H, methoxyl), 2.83 (s, 3H, 6' and 17-H), 1.00 (s, 3H, 19-CH_3), 0.93 (s, 3H, 18-CH_3).

Acid Treatment of Compound 10

The dihydro compound 10 (1 g) was dissolved in methanol (50 ml) and stirred with 10% oxalic acid solution (10 ml) till all of the dihydro compound was converted to the β,γ-unsaturated compound (30 min). The stirring was continued for 8 h after which all of the β,γ-unsaturated compound was converted to the most stable α,β-unsaturated compound 11. Methylene chloride was added into the flask and the organic layer was washed with saturated sodium bicarbonate, water, brine, and dried over anhydrous magnesium sulphate. The solvent was evaporated *in vacuo* and the residue was chromatographed on preparative silica gel plate (ether:hexane; 1:1) to give the α,β-unsaturated compound 11 as an oil (600 mg, 62.5%). Spectral analysis of β,γ-unsaturated compound: Ir ($CHCl_3$): 3625 (hydroxyl), 1710 cm^{-1}(carbonyl). Nmr ($CDCl_3$): δ 5.76 (broad s, 1H, vinylic, 4'H), 4.13 (broad s, 1H, 3α-H), 2.90 (broad, 2H, 2'allylic H), 2.39 (s, 3H, 5'H and 17α-H), 0.96 (s, 3H, 19-CH_3), 0.83 (s, 3H, 18-CH_3).

Spectral analysis of compound 11: Ir ($CHCl_3$): 3625 cm^{-1} (hydroxyl), 1660 cm^{-1} (carbonyl). Nmr ($CDCl_3$): δ 5.96 (s, 1H, vinylic 2'H), 4.13 (broad s, 1H, 3α-H), 0.96 (s, 3H, 19-CH_3), 0.86 (s, 3H, 18-CH_3). Uv λ_{max}^{EtOH}: 245, A_{245} = 0.2275, ε = A/Cl = 8781.5. Anal. calcd for $C_{25}H_{38}O_3$: C, 77.72; H, 9.84; O, 12.43. Found: C, 77.64; H, 9.76; O. 12.37. Ms: m/z 386.

5β-Androstan-17β-(1'-oxocyclohex-2'-en-3'-yl)-3β,14β-diol 3-(2", 3", 4", 6")-tetra-O-acetyl-β-D-glucopyranoside 12

Compound 11 (1.2 g) in distilled 1,2-dichloroethane (50 ml) was stirred with dry silver oxide (2.88 g) and anhydrous magnesium sulphate (5.76 g) at room temperature. After 1 h, aceto-α-bromo-D-glucose (3.82 g) in 1,2-dichloroethane (5 ml) was added dropwise and the stirring continued for 48 h . The reaction mixture was filtered through a celite pad and washed with chloroform. The filtrate was evaporated to dryness and the residue was chromatographed on the thin layer chromatography plate (silica gel, ether:hexane; 1:4) to give the pure acetate 12 as an oil (800 mg) and the starting material 11 (350 mg). Ir ($CHCl_3$): 3625 cm^{-1} (hydroxyl), 1800 cm^{-1} (acetate carbonyl), 1700 cm^{-1} (α,β-carbonyl). Nmr ($CDCl_3$): δ 5.96 (s, 1H, 2'H), 5.16-3.46 (m, 5H, 2",3",4",& 6" H), 2.09 and 2.03 (each singlets, 12H, CH_3CO × 4), 0.89 (s, 3H, 19-CH_3), 0.86 (s, 3H, 18-CH_3).

5β-Androstan-17β-(1'-oxocyclohex-2'-en-3'-yl)-3β,14β-diol 3-β-D-glucopyranoside 13

The acetate 12 (850 mg) in dry methylene chloride (8 ml) and methanol (10 ml) was stirred with saturated ammonia in methanol (100 ml) for 5 min and was kept stored for 16 h at 4°C. After the reaction was finished, the solvent was evaporated in vacuo and the residue was chromatographed on preparative silica gel plate (methanol:ether; 1:4) to furnish the crystalline compound 13 (590 mg, mp 230-32°C, 90.7%). Ir (KBr pellet): 3425 cm^{-1} (hydroxyl), 1760 cm^{-1} (α,β-carbonyl). Nmr ($CDCl_3$ + DMSO-d_6): δ 5.89 (s, 1H, 2'H), 4.68-3.51 (m, OH), 0.79 (s, 3H, 18-CH_3), 0.89 (s, 3H, 19-CH_3). Anal. calcd for $C_{31}H_{48}O_8$: C, 67.88; H, 8.75; O, 23.35. Found: C, 67.81; H, 8.70; O, 23.29. Ms: m/z 548.

REFERENCES

1. T.Y.R. Tsai, A. Minta, and K. Wiesner, Heterocycles, 1979, 12, 1397.
2. R. Mendez, G. Pastlin, and E. Kabala, J. Pharmacol. Exp. Ther., 1974, 188, 189; J.I. Gliklich, R. Gaffney, M.R. Rosen, and B.F. Hoffman, Eur. J. Pharmacol., 1975, 32, 1; C.R. Ross and N.I. Pessah, Eur.J. Pharmacol., 1975, 33, 223; G. T. Okita, Federation Proceedings., 1977, 36, 2225.
3. R. Thomas, J. Boutagy, and A. Gelbert, J. Pharma. Sci., 1974, 63, 1649; J. Boutagy, A. Gelbert, and R. Thomas, Aus. J. Pharm. Sci., 1973, 2, 41.
4. K. Wiesner and T.Y.R. Tsai, Pure and Appl. Chem., 1986, 58, 799.
5. T. Minesita, R. Hirota, S. Kimoto, M. Uno, and Y. Uemura, Ann. Rep. Shionogi Res. Lab., 1968, 18, 94; T. Nambara, K. Shimada, T. Nemoto, and S. Goya, Chem. Pharm. Bull., 1970, 18, 1658; P. Smith, L. Brown, J. Boutagy, and R. Thomas, J. Med. Chem., 1982, 25, 1222.
6. R. Contreras and L. Mendoza, Steroids, 1979, 34, 121.
7. R.G. Harvey, Synthesis, 1970, 161.
8. C.R. Engel and G. Bach, Steroids, 1964, 3, 593.
9. For a review, see A.J. Birch and S. Rao, Adv. Org. Chem., 1972, 8, 1.
10. J-M. Ferland and L. Yvon, Chem. Abs., 1969, 71, 113228.

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