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IDENTIFICATION AND SYNTHESIS OF A METABOLITE OF KH 1060, A NEW POTENT 1α,25-DIHYDROXYVITAMIN D_3 ANALOGUE

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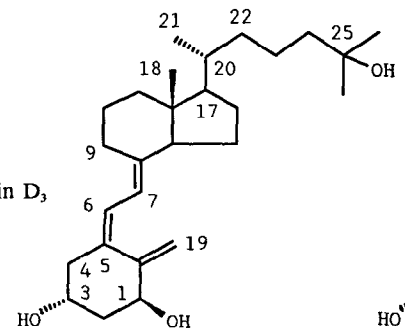
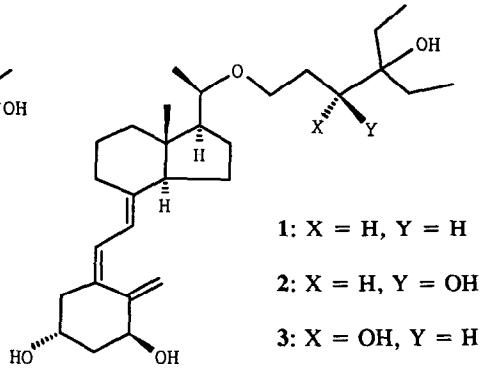
Abstract: A metabolite (**2**) from pig liver of a potent analogue (KH 1060, **1**) of 1α,25-dihydroxyvitamin D_3 is identified by spectroscopy and chemical synthesis starting from (*S*)-malic acid.

The recent report[1] that KH 1060 (**1**), a side chain modified analogue of 1α,25-dihydroxyvitamin D_3, appears to be one of the most potent *in vitro* regulators of cell growth, differentiation and cytokine-mediated T-lymphocyte activation studied so far prompted us to investigate the metabolism of **1**.

Thus, the metabolite with the major UV-absorbing (264 nm) HPLC peak from the treatment of **1** with post-mitochondrial supernatant from pig liver was isolated.[2] Mass and ^{1}H-NMR[3] spectral analysis[4] led us to propose that the metabolite had one of two possible structures, **2** and **3**. Conclusive identification was achieved through the chemical synthesis of **2** and **3**, reported below.[3]



1α,25-Dihydroxyvitamin D_3



1: X = H, Y = H

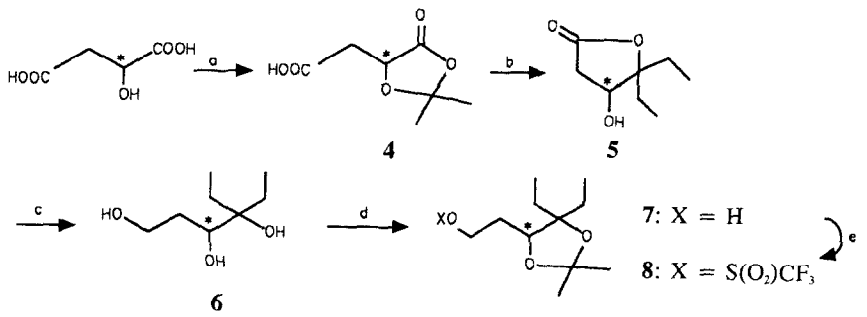
2: X = H, Y = OH

3: X = OH, Y = H

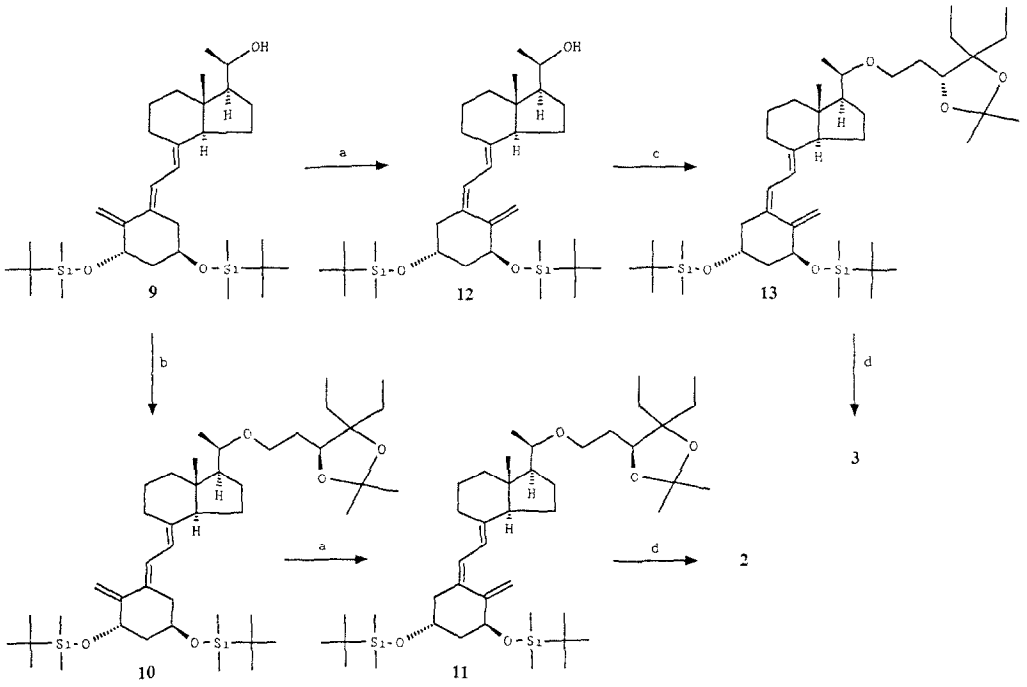
Compound **1** and other 20-epi-22-oxa analogues of 1α,25-dihydroxyvitamin D_3 with monohydroxylated side chains have been prepared[5] by alkylation of the 20(*R*)-alcohol **9** with suitable side chain fragments followed by 5,6-double bond isomerization and then finished with a deprotection of all alcohol groups. This as well as an alternative sequence of reactions were employed in the synthesis of **2** and **3**, described below.

All reactions depicted in Scheme 1 were carried out in both the (*S*)- and the (*R*)-series, starting from (*S*)- and (*R*)-malic acid, respectively: Addition of the 1,3-dioxolan-5-one (**4**), prepared from malic acid,[6] to ethyl magnesium bromide followed by treatment with hydrochloric acid gave the hydroxybutyrolactone (**5**).[7] Reduction of **5** with $LiAlH_4$ produced the triol **6**.[8] The 1,2-diol system was protected with acetone to give **7**.[9,10] The tosylate of (*S*)-**7** as well as the corresponding bromide were unreactive in alkylation of **9** (*cf.* ref. 5) whereas the highly reactive triflate (*S*)-**8**[11] alkylated the alcohol **9** (Scheme 2) to give **10**, albeit in low yield.[12] 5,6-Double bond isomerization[13] followed by deprotection with HF/CH_3CN gave one of the desired vitamin D analogues, **2**.[14] Alkylation of the alcohol **12**, obtained from 5,6-double bond isomerization of compound **9**, with (*R*)-**8**[15] followed by deprotection gave the second desired vitamin D analogue, **3**.[16]

The ^{1}H- as well as the ^{13}C-NMR spectra of **2** and **3** are clearly different and the ^{1}H NMR spectrum of compound **2**, synthesized from *(S)*-malic acid, clearly established its identity with the KH 1060 metabolite from pig liver. Furthermore, no cross-contamination was observed which means that no detectable racemization/epimerization at the chiral carbon atom bearing the secondary OH-group of the side chain has taken place during the synthesis from (*S*)- and (*R*)-malic acid to **2** and **3**, respectively.



Scheme 1. The reactions were carried out with either (*R*)- or (*S*)-malic acid as starting material. (a) $Me_2C(OMe)_2$/PPTS, 60% (ref. 6); (b)(1) EtMgBr/THF/10-15°C/50 min, (2) 1M HCl to pH 1/r.t./1h, (3) chromatography (silica gel, Et_2O), 40%; (c) $LiAlH_4$/THF/reflux/1h, 75%; (d) Me_2CO/*p*-TsOH, 88%; (e) $(CF_3SO_2)_2O$/pyridine/CH_2Cl_2/0°C/20 min (see note 11).



Scheme 2. (a) hν/anthracene/Et_3N/CH_2Cl_2, 70-80%; (b) KH/18-crown-6/(*S*)-**8**/THF/25°C, 34%; (c) KH/18-crown-6/(*R*)-**8**/THF/25°C, 16%; (d) HF/CH_3CN/EtOAc/H_2O/r.t./1h, ca. 40%.

The biological hydroxylation of **1** is reminiscent of the side chain hydroxylation of 25-hydroxyvitamin D_3 and 1α,25-dihydroxyvitamin D_3, where 24*R*-hydroxylation occurs (*cf.* ref. 17). More details on the metabolism of KH 1060 will be reported elsewhere.[2]

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References and notes

1. Binderup, L.; Latini, S.; Binderup, E.; Bretting, C.; Calverley, M.; Hansen, K. *Biochem. Pharmacol.*, **1991**, *42*, 1569-1575.
2. Kissmeyer, A.-M.; Sørensen, H. *Xenobiotica*, submitted.
3. NMR spectra were recorded in $CDCl_3$ at 300 MHz (^1H) and 75 MHz (^{13}C). Chemical shifts are given in ppm relative to TMS at 0.00 ppm. Coupling constants are given in Hz.
4. Spectral data of the metabolite: HR-MS: calcd ($C_{29}H_{48}O_5$) 476.350, found 476.350; ^1H NMR δ: 0.56 (s, 3H, H-18), 0.88 (t, 6H, 2x $CH_2C\underline{H}_3$), 1.10 (d, 3H, H-21), 1.20 - 2.25 (m, 23H), 2.31 (dd, 1H, H-4), 2.60 (dd, 1H, H'-4), 2.84 (bd, 1H, H-9), 3.33 (m, 1H, H-20), 3.51 (m, 1H, OC$\underline{H}$H'), 3.71 (m, 1H, O-C$\underline{H}$-C-O), 3.77 (m, 1H, OCH$\underline{H}$'), 4.23 (m, 1H, H-3), 4.42 (m, 1H, H-1), 5.00 (bs, 1H, H-19), 5.32 (m, 1H, H'-19), 6.00 (d, J=11.2, 1H, H-7), 6.38 (d, J=11.2, 1H, H-6).
5. Hansen, K.; Calverley, M. J.; Binderup, L. *Vitamin D: Gene Regulation, Structure-Function Analysis and Clinical Application*; Norman, A. W.; Bouillon, R.; Thomasset, M., Eds.; Walter de Gruyter: Berlin, 1991; pp. 161-162.
6. Sterling, J.; Slovin, E.; Barasch, D. *Tetrahedron Lett.*, **1987**, *28*, 1685-1688.
7. To a solution of ethyl magnesium bromide (280 mmol) in THF (120 mL) at 10-15°C was added over 20 min a solution of compound **4**[6] (6.06 g, 35 mmol) in dry THF (60 mL). Stirring (1 h, 10°C), acidification with 1M hydrochloric acid (265 mL), stirring (1 h, 22°C), neutralization with aq NaOH, extraction with ether, drying and evaporation of solvent gave an oil which was chromatographed on silica gel with ether to give **5** (2.23 g, 14 mmol, 40%) as a pale yellow oil. (*S*)-**4** gave (*S*)-**5**: ^1H-NMR δ: 0.95 (t, 3H, C$\underline{H}_3$), 0.98 (t, 3H, C'$\underline{H}_3$), 1.64 (m, 2H), 1.85 (m, 2H), 2.52 (dd, J=3.1, J=18.3, 1H, C$\underline{H}$H'C=O), 2.94 (dd, J=6.7, J=18.3, 1H, CH$\underline{H}$'C=O), 4.30 (dd, J=6.7, J=3.1, 1H, C$\underline{H}$OH), 3.00 (broad line, 1H, -O$\underline{H}$); ^{13}C-NMR δ: 7.73 ($\underline{C}H_3$), 7.86 ($\underline{C}'H_3$), 23.40, 28.10, 38.82 ($\underline{C}H_2C$=O), 71.73 ($\underline{C}$HOH), 92.89 ($\underline{C}$(Et)$_2$), 175.67 ($\underline{C}$=O); $[\alpha]^{20}_D$ -8.8° (1.9, $CHCl_3$); Anal calcd for $C_8H_{14}O_3$: C, 60.74%; H, 8.92%; found: C, 60.65%; H, 9.31%; MS (CI, 2-methylpropane) 159 ($M+H^+$), 141 ($M+H^+-H_2O$, base peak). (*R*)-**4** gave (*R*)-**5**, which crystallized on standing: Mp 34-35°C; NMR and MS data as for (*S*)-**5**; $[\alpha]^{20}_D$ +8.1° (2.0, $CHCl_3$); Anal calcd as for (*S*)-**5**; found: C, 60.55%; H, 9.02%.
8. Compound **5** (4.1 g, 25 mmol) in dry THF (25 mL) was slowly added to $LiAlH_4$ (1.2 g, 30 mmol) in dry THF (25 mL) under argon. Reflux for 1 h, addition of satd aq NH_4Cl (40 mL), extraction with EtOAc and evaporation of solvent gave after chromatography (silica gel, ether followed by ether/MeOH 1:1 as eluent) the triol **6** (3.14 g, 19.4 mmol, 75%) as a colourless oil. (*S*)-**5** gave (*S*)-**6**: ^1H-NMR δ: 0.88 (t, 6H, 2x C$\underline{H}_3$), 1.39 (m, 1H), 1.60 (m, 4H), 1.71 (m, 1H), 2.98 (bs, 1H, -O$\underline{H}$), 3.72 (q, 1H, C$\underline{H}$OH), 3.83 (m, 2H, C$\underline{H}_2$OH), 3.95 (bd, 1H, -O$\underline{H}$), 4.10 (bs, 1H, -O$\underline{H}$); ^{13}C-NMR δ: 7.25 ($\underline{C}H_3$), 7.41 ($\underline{C}'H_3$), 25.95, 26.96, 32.07 (O-C-$\underline{C}H_2$-C-O), 60.73 ($\underline{C}H_2$OH), 74.08 ($\underline{C}$HOH), 76.06 (*tert*-$\underline{C}$-OH); $[\alpha]^{20}_D$ -25.9° (2.0, EtOH); FAB-MS: calcd for ($MH^+ = C_8H_{19}O_3^+$) 163.1334, found 163.1327. (*R*)-**5** gave (*R*)-**6**: NMR spectra as for (*S*)-**6**; $[\alpha]^{20}_D$ +26.8° (2.0, EtOH); FAB-MS: calcd as for (*S*)-**6**; found 163.1314.
9. A solution of **6** (2.0 g, 12 mmol) and *p*-toluenesulfonic acid (60 mg) in acetone (85 mL) was left for 22 h at 22°C and then stirred with solid $NaHCO_3$ (2 g) for 10 min. Evaporation of solvent *in vacuo*, addition of EtOAc (100 mL) to the residue, wash with satd aq $NaHCO_3$ and satd aq NaCl, drying and evaporation of solvent *in vacuo* gave **7** (2.1 g, 10.5 mmol, 88%) as an oil. (*S*)-**6** gave (*S*)-**7**: ^1H-NMR δ: 0.89 (t, 3H, $CH_2C\underline{H}_3$), 0.92 (t, 3H, $CH'_2C'\underline{H}_3$), 1.35 (m, 1H), 1.36 (s, 3H, O-C-C$\underline{H}_3$), 1.42 (s, 3H, O-C-C'$\underline{H}_3$, 1.62 (m, 4H), 1.87 (m, 1H), 2.45 (dd, 1H), 3.83 (m, 2H, C$\underline{H}_2$O), 4.01 (dd, J=2.28, J=10.7, 1H, dioxolane-C$\underline{H}$); ^{13}C-NMR δ: 6.88 ($CH_2\underline{C}H_3$), 7.82 ($C'H_2\underline{C}'H_3$), 25.20, 26.41 (O-C-$\underline{C}H_3$), 26.86, 28.04 (O-C-$\underline{C}'H_3$), 31.52 ($\underline{C}H_2CH_2O$), 61.27 ($\underline{C}H_2O$), 80.13 (O-$\underline{C}$H), 83.92 ($\underline{C}$(Et)$_2$), 106.62 ($\underline{C}$(CH$_3$)$_2$); $[\alpha]^{20}_D$ -8.1° (2.0, EtOH); EI-MS: calcd for ($M-15^+ = C_{10}H_{19}O_3^+$) 187.1334, found 187.1325. (*R*)-**6** gave (*R*)-**7**: NMR spectra as for (*S*)-**7**; $[\alpha]^{20}_D$ +8.0° (2.0, EtOH); EI-MS: calcd as for (*S*)-**7**; found 187.1319.

10. The initially formed six-membered acetonide of the 1,3-diol system (TLC, hexane/EtOAc, R_f 0.4) was isomerized to the five-membered acetonide (R_f 0.3) under the reaction conditions with only a trace of the six-membered acetonide in the final mixture (*cf.* ref. 6).
11. A solution of **7** (650 mg, 1.8 mmol) and pyridine (145 µl, 1.8 mmol) in CH_2Cl_2 (20 mL) was slowly added under argon to a solution of trifluoromethanesulfonic anhydride (584 mg, 2.1 mol) in CH_2Cl_2 (20 mL) at 0°C. After stirring for 20 min CH_2Cl_2 (10 mL) was added and the mixture was quickly washed with water (10 mL). Drying ($MgSO_4$ + $NaHCO_3$) and evaporation of solvent over a few mg of $NaHCO_3$ gave **8** as an oil which was, due to instability, immediately used in the following reaction. (*S*)-**7** gave (*S*)-**8**: ^{1}H-NMR δ: 0.89 (t, 3H, $CH_2C\underline{H}_3$), 0.93 (t, 3H, $CH'_2C'\underline{H}_3$), 1.34 (s, 3H, O-C-$C\underline{H}_3$), 1.41 (s, 3H, O-C-$C'\underline{H}_3$), 1.60 (m, 4H, 2x $C\underline{H}_2CH_3$), 1.97 (m, 2H, $OCH_2C\underline{H}_2$), 3.96 (dd, J=3.6, J=9.4, 1H, dioxolane-C$\underline{H}$), 4.71 (m, 2H, $C\underline{H}_2O$); ^{13}C-NMR δ: 7.31, 8.01, 25.60, 26.91, 27.78, 28.47, 29.96, 75.24, 76.61, 84.04 (O-$\underline{C}$-CH-O), 107.45 (O-$\underline{C}$-O), 118.78 (q, J=319.7, -$\underline{C}F_3$). (*R*)-**7** gave (*R*)-**8**: no spectral data.
12. Compound **10**: ^{1}H-NMR δ: 0.05 (bs, 12H), 0.57 (s, 3H, H-18), 0.87 (s, 9H), 0.90 (m, 6H, 2x $CH_2C\underline{H}_3$), 0.91 (s, 9H), 1.10 (d, 3H, H-21), 1.10-1.87 (m, 16H), 1.34 (s, 3H, O-C-$C\underline{H}_3$), 1.42 (s, 3H, O-C-$C'\underline{H}_3$), 1.94 (m, 1H), 2.05 (t, 1H), 2.15 (d, 1H), 2.31 (d, 1H, H-4), 2.54 (dd, 1H, H'-4), 2.90 (dd, 1H, H-9), 3.34 (m, 2H, $OC\underline{H}_2$), 3.64 (q, 1H, H-20), 3.98 (dd, 1H, dioxolane-C$\underline{H}$), 4.23 (m, 1H, H-3), 4.54 (m, 1H, H-1), 4.95 (bs, 1H, H-19), 5.00 (bs, 1H, H'-19), 5.81 (d, J=11.2, 1H, H-7), 6.47 (d, J=11.2, 1H, H-6); ^{13}C-NMR δ: -5.07, -4.95, 7.13, 7.94, 12.45, 17.88, 18.03, 18.10, 22.28, 23.36, 24.98, 25.44, 25.62, 25.67, 26.70, 27.02, 28.32,28.67, 30.28, 36.46, 40.26, 43.83, 45.69, 55.83, 56.75, 65.39, 67.07, 70.15, 77.84, 78.05, 83.79, 106.24, 106.47, 116.17, 121.54, 135.10, 143.13, 153.44 .
13. Compound **11**: ^{1}H-NMR δ: 0.07 (s, 6H), 0.07 (s, 6H), 0.56 (s, 3H, H-18), 0.90 (s, 18H), 0.91 (t, 6H, 2x $CH_2C\underline{H}_3$), 1.09 (d, 3H, H-21), 1.10 - 1.90 (m, 13H), 1.34 (s, 3H, O-C-$C\underline{H}_3$), 1.42 (s, 3H, O-C-$C'\underline{H}_3$), 1.63 (q, 4H, 2x $C\underline{H}_2CH_3$), 2.01 (t, 1H, H-17), 2.15 (bd, 1H), 2.23 (dd, 1H, H-4), 2.46 (dd, 1H, H'-4), 2.84 (bd, 1H, H-9), 3.30 (m, 1H, OC$\underline{H}$H'), 3.39 (m, 1H, OCH$\underline{H}$'), 3.64 (q, 1H, H-20), 3.99 (dd, J=2.4, J=9.8, 1H, dioxolane-C$\underline{H}$), 4.20 (m, 1H, H-3), 4.38 (m, 1H, H-1), 4.87 (d, J=2.4, 1H, H-19), 5.19 (m, 1H, H'-19), 5.81 (d, J=11.2, 1H, H-7), 6.25 (d, J=11.2, 1H, H-6); ^{13}C-NMR δ: -5.26, -4.98, -4.87, 7.12, 7.95, 12.40, 17.94, 18.03, 18.11, 22.17, 23.30, 24.97, 25.38, 25.62, 25.66, 25.70, 26.70, 26.92, 28.31, 28.77, 30.23, 40.26, 44.64, 45.54, 45.85, 55.66, 56.64, 65.43, 67.32, 71.88, 77.90, 78.00, 83.80, 106.25, 110.94, 117.59, 122.96, 134.73, 140.85, 148.16.
14. Compound **2**: MS and ^{1}H-NMR spectral data, see note 4. ^{13}C-NMR δ: 7.28, 7.47, 12.58, 18.32, 22.17, 23.33, 24.78, 26.10, 27.36, 28.90, 30.92, 40.02, 42.75, 45.12, 45.54, 55.60, 56.52, 66.24, 66.63, 70.84, 73.83, 75.65, 78.22, 111.51, 116.90, 124.70, 132.86, 142.68, 147.55.
15. For alkylation procedure, see ref. 5. Compound **13**: ^{1}H-NMR δ: 0.05 (bs, 12H), 0.58 (s, 3H, H-18), 0.86 (s, 18H), 0.90 (t, 6H), 1.10 (d, 3H, H-21), 1.15 - 1.95 (m, 17H), 1.32 (s, 3H, O-C-$C\underline{H}_3$), 1.41 (s, 3H, O-C-$C'\underline{H}_3$), 2.01 (t, 1H, H-17), 2.19 (m, 2H), 2.45 (dd, 1H, H-4), 2.84 (bd, 1H, H-9), 3.32 (m, 2H, OC$\underline{H}$H' and H-20), 3.75 (m, 1H, OCH$\underline{H}$'), 4.14 (dd, 1H, dioxolane-C$\underline{H}$), 4.20 (m, 1H, H-3), 4.38 (m, 1H, H-1), 4.87 (d, J=2.4, 1H, H-19), 5.18 (bs, 1H, H'-19), 6.01 (d, J=11.2, 1H, H-7), 6.25 (d, J=11.2, 1H, H-6); ^{13}C-NMR δ: -5.26, -4.98, -4.87, 7.14, 7.94, 12.31, 18.02, 22.22, 23.24, 25.20, 25.66, 26.79, 26.89, 28.32, 28.70, 30.18, 32.59, 40.32, 44.64, 45.53, 45.85, 55.71, 56.73, 64.90 ($O\underline{C}H_2$), 67.32 (C-3), 71.89 (C-1), 77.35, 78.30, 83.71 (O-$\underline{C}$-CH-O), 106.26 (O-$\underline{C}$-O), 110.94 (C-19), 117.63, 122.92, 134.80, 140.74, 148.16.
16. Compound **3**: EI-MS: calcd ($C_{29}H_{48}O_5$) 476.350, found 476.347. ^{1}H-NMR δ: 0.55 (s, 3H, H-18), 0.87 (t, 6H, 2x $CH_2C\underline{H}_3$), 1.13 (d, 3H, H-21), 1.15 - 2.15 (m, 19H), 1.57 (q, 4H, 2x $C\underline{H}_2CH_3$), 2.31 (dd, 1H, H-4), 2.60 (dd, 1H, H'-4), 2.83 (dd, 1H, H-9), 3.28 (m, 1H, H-20), 3.51 (m, 1H, OC$\underline{H}$H'), 3.71 (bd, 1H, O-C$\underline{H}$-C-O), 3.80 (bt, 1H, OCH$\underline{H}$'), 4.24 (m, 1H, H-3), 4.43 (m, 1H, H-1), 4.99 (s, 1H, H-19), 5.32 (s, 1H, H'-19), 6.00 (d, J=11.2, 1H, H-7), 6.38 (d, J=11.2, 1H, H-6); ^{13}C-NMR δ: 7.27, 7.64, 12.53, 18.00, 22.17, 23.32, 24.81, 25.89, 27.00, 28.87, 29.94, 40.14, 42.65, 45.03, 45.55, 55.50, 56.74, 66.58, 67.13, 70.58, 75.10, 75.35, 78.64, 111.53, 116.86, 124.67, 132.83, 142.61, 147.45.
17. Jones, G.; Vriezen, D.; Lohnes, D.; Palda, V.; Edwards, N. S. *Steroids*, **1987**, *49*, 29-53.