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TWO NEW 4α-METHYLSTEROLS IN THE SEEDS OF *BRASSICA NAPUS*

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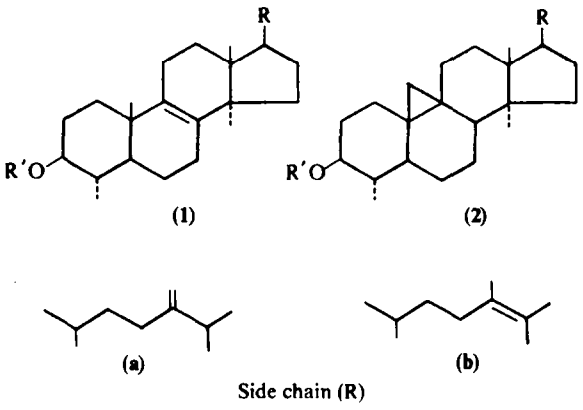
Key Word Index—*Brassica napus*; Cruciferae; 4α-methylsterols; 4α,14α,24-trimethylcholesta-8,24-dien-3β-ol; 4α,14α,24-trimethyl-9β,19-cyclocholest-24-en-3β-ol; seeds.

Our previous paper described the occurrence of 24-methylenelanost-8-en-3β-ol in the 4,4-dimethylsterol fraction from the seeds of *Brassica napus* [1]. We now report the detection and tentative identification of two new sterols in the 4-monomethylsterol fraction separated from the seeds. 4α-Methylsterols hitherto found in the 4-monomethylsterol fraction from the Cruciferous seeds are 31-norlanostenol and/or 4α-methylzymostenol [2], lophenol [3], 31-norlanosterol [2], obtusifoliol [2, 4], gramisterol (24-methylenelophenol) [2, 4] and citrostadienol [2, 4, 5].

The 4-monomethylsterol fraction separated from the unsaponifiable matter of the seeds of *B. napus* [1] was acetylated with Ac_2O–Py. The acetate fraction (108 mg) obtained was separated into six bands by PLC on $AgNO_3$–Si gel (1:4) with two developments using CCl_4–CH_2Cl_2 (5:1). The fraction (3 mg) recovered from the second band (R_f 0.47) from the solvent front gave two peaks on GLC. GC–MS of the faster eluted major component (A, 68%) showed that it was an acetate of a C_{30} sterol with two double bonds (*m/e* 468, M^+, $C_{32}H_{52}O_2$) of which one was present in the side chain (*m/e* 341, $M^+ - C_9H_{17}$ [SC] − 2H). The fragment ions at *m/e* 301 ($M^+ - SC - C_3H_6$) and 287 (*m/e* 301 − CH_2) indicated the presence of an additional C-32 methyl group in the ring system. Furthermore, the fragment ions at *m/e* 384 ($M^+ - C_6H_{12}$) and 369 (*m/e* 384 − Me) showed that the side chain double bond was located either at C-24(28) or at C-24(25) [6]. The *RR*, of the steryl acetate was 1.82 (SE-30), 1.74 (Dexil-300), 1.76 (OV-17) and 1.79 (OV-210). Since the data from MS, GLC and argentation TLC were identical with those of authentic 4α,14α,24-trimethyl-5α-cholesta-8,24-dien-3β-yl acetate (**1b**, R′ = Ac) prepared from obtusifoliyl acetate (**1a**, R′ = Ac), the steryl acetate A is considered to have the structure **1b**, R′ = Ac.

GC–MS of the slower eluted minor component (B, 32%) showed that it was also an acetate of a C_{30} sterol with two double bonds (*m/e* 468, M^+). The fragment ions at *m/e* 341, 384 and 369 and at *m/e* 301 and 287 indicated the presence of a double bond located at C-24(28) or at C-24(25) in the side chain and an additional C-32 methyl group in the ring system, respectively. Furthermore, the ion at *m/e* 285 ($M^+ - C_{10}H_{16}O_2$ − Me) was probably due to the presence of 9β,19-cyclopropyl group rather than the double bond in the ring system [7]. The *RR*, of the steryl acetate was 2.09 (SE-30), 2.05 (Dexsil-300), 2.09



(OV-17) and 2.05 (OV-210). The data from MS, GLC and argentation TLC were in good agreement with those of authentic 4α,14α,24-trimethyl-9β,19-cyclo-5α-cholest-24-en-3β-yl acetate (**2b**, R′ = Ac) prepared from cycloeucalenyl acetate (**2a**, R′ = Ac). Thus, the steryl acetate B is considered to have the structure **2b**, R′ = Ac.

Though isomerisation of a sterol with the $\Delta^{24(28)}$ side chain (R = a) to a $\Delta^{24(25)}$ side chain (R = b) during chromatography on Si gel has been recognized [8], a careful preliminary experiment using 24-methylenecycloartanol (R = a) under the same conditions used in this study showed no detectable formation of cyclobranol (24-methylcycloart-24-enol, R = b). Therefore, the two steryl acetates A and B detected in the acetylated 4-monomethylsterol fraction from the seeds of *B. napus* are considered to be natural products rather than artefacts.

There are four 24-alkylated Δ^{24}-sterols hitherto reported to occur in higher plants: cyclobranol as its ferulate in rice-bran oil [9], 24-ethylcholesta-7,24-dien-3β-ol in sunflower oil [10], and 24-methylcholesta-5,24-dien-3β-ol and its 24-ethyl homolog in *Withania somnifera* (Solanaceae) [11]. This work appears to be the first record of the detection of 4α-methyl 24-alkylated Δ^{24}-sterols (**1b** and **2b**) in higher plants. The biogenetic importance of the 24-alkylated Δ^{24}-sterols has been well discussed [11–16].

EXPERIMENTAL

Recrystallizations were performed in Me_2CO–MeOH. Mp's are uncorr. IR spectra were recorded in KBr. PMR spectra were measured at 100 MHz in $CDCl_3$ with TMS as internal reference. MS (70 eV, >*m/e* 200) were taken with a GC–MS (2% OV-17 column) or with a probe injection. GLC analyses (chloesteryl acetate, *RR*, = 1.00) were carried out on 2 m × 3 mm glass columns packed with 2% SE-30 (column temp. 228°), 2% Dexil-300 (255°), 3% OV-17 (265°) and 2% OV-210 (202°) on 80–100 mesh Gas Chrom-Z, respectively, with 50 ml/min N_2.

Obtusifoliyl acetate (**1a**, R′ = Ac). Obtusifoliol (**1a**, R′ = H) was isolated from the latex of *Euphorbia regis-Jubae* W. B. as described by González *et al.* [17]. Its acetate (**1a**, R′ = Ac) showed mp 106–108° (lit. [17] mp 107–108°). IR ν_{max} cm^{-1}: 3080, 1638, 880 (C=CH_2), 1730, 1246 (OAc). PMR δ: 0.71 (3H, *s*, C-18), 0.89 (3H, *s*, C-32), 0.99 (3H, *s*, C-19), 2.05 (3H, *s*, 3β-OAc), 0.86 (3H, *d*, *J* = 6.3 Hz, C-30), 0.92 (3H, *d*, *J* = 6.9 Hz, C-21), 1.02 (6H, *d*, *J* = 7.6 Hz, C-26, 27), 4.38 (1H, *m*, $W_{1/2}$ = 27 Hz, 3α-H), 4.69 (2H, *bd*, *J* = 4.4 Hz, C-28). MS (probe) *m/e* (rel. int.): 468 (M^+, $C_{32}H_{52}O_2$, 60), 453 (100), 408 (4), 393 (24), 369 (9), 341 (2), 324 (3), 301 (9), 287 (22), 227 (20).

4α,14α,24-Trimethyl-5α-cholesta-8,24-dien-3β-yl acetate (**1b**, R′ = Ac). Isomerisation of **1a**-acetate (50 mg) with H_2SO_4 in IPA and EtOAc under reflux [18] yielded a mixture of dienes differing in the position of the side chain double bond. Separation of this mixture by $AgNO_3$–Si gel PLC gave **1b**-acetate (6 mg), mp 139–140°. IR ν_{max} cm^{-1}: 1730, 1246 (OAc). PMR δ: 0.70 (3H, *s*, C-18), 0.89 (3H, *s*, C-32), 0.98 (3H, *s*, C-19), 1.63 (9H, *s*, C-26, 27, 28), 2.05 (3H, *s*, 3β-OAc), 0.85 (3H, *d*, *J* = 5.9 Hz, C-30), 0.93 (3H, *d*, *J* = 6.0 Hz, C-21), 4.38 (1H, *m*, $W_{1/2}$ = 26 Hz, 3α-H). MS (probe) *m/e* (rel. int.): 468 (M^+, $C_{32}H_{52}O_2$, 55), 453 (100), 408 (3), 393 (29), 384 (4), 369 (11), 341 (2), 301 (4), 287 (8), 227 (10).

Cycloeucalenyl acetate (**2a**, R′ = Ac). Cycloeucalenol (**2a**, R′ = H) was isolated from the unsaponifiable matter, obtained by saponification of the ferulate fraction separated from rice-bran oil, by PLC as described previously [19]. The alcohol on acetylation (Ac_2O–Py) yielded the acetate (**2a**, R′ = Ac), mp 108–110° (lit. [19] mp 110–111°). IR ν_{max} cm^{-1}: 3080, 1638, 885 (C=CH_2); 3040, 1018 (cyclopropyl), 1730, 1240 (OAc), PMR δ: 0.90 (3H, *s*, C-32), 0.98 (3H, *s*, C-18), 2.05 (3H, *s*, 3β-OAc), 0.16 (1H, *d*, *J* = 4.2 Hz, C-19), 0.42 (1H, *d*, *J* = 4.2 Hz, C-19), 0.86 (3H, *d*, *J* = 5.7 Hz, C-30), 1.03 (6H, *d*, *J* = 6.0 Hz, C-26, 27), 4.71 (2H, *bd*, *J* = 4.3 Hz, C-28), 4.56 (1H, *m*, $W_{1/2}$ = 26 Hz, 3α-H). MS (probe) *m/e* (rel. int.): 468 (M^+, $C_{32}H_{52}O_2$, 7), 453 (8), 408 (100), 393 (51), 384 (5), 365 (7), 343 (5), 300 (10), 285 (3), 283 (13).

4α,14α,24-Trimethyl-9β,19-cyclo-5α-cholest-24-en-3β-ol (**2b**, R′ = Ac). Isomerisation of **2a**-acetate with H_2SO_4 as described above yielded **2b**-acetate, mp 159–160°. IR ν_{max} cm^{-1}: 3040, 1020 (cyclopropyl), 1732, 1240 (OAc). PMR δ: 0.89 (3H, *s*, C-32), 0.97 (3H, *s*, C-18), 1.63 (9H, *s*, C-26, 27, 28), 2.05 (3H, *s*, 3β-OAc), 0.17 (1H, *d*, *J* = 4.4 Hz, C-19), 0.42 (1H, *d*, *J* = 4.4 Hz, C-19), 0.86 (3H, *d*, *J* = 7 Hz, C-30), 0.91 (3H, *d*, *J* = 7 Hz, C-21), 4.52 (1H, *m*, $W_{1/2}$ 25 Hz, 3α-H). MS (probe) *m/e* (rel. int.): 468 (M^+, $C_{32}H_{52}O_2$, 19), 453 (7), 408 (100), 393 (59), 384 (2), 369 (1), 353 (7), 341 (2), 309 (5), 301 (2), 287 (1), 285 (5), 227 (3).

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EIN NEUES *P*-HYDROXYACETOPHENON-DERIVAT AUS *ARTEMISIA MONOSPERMA*

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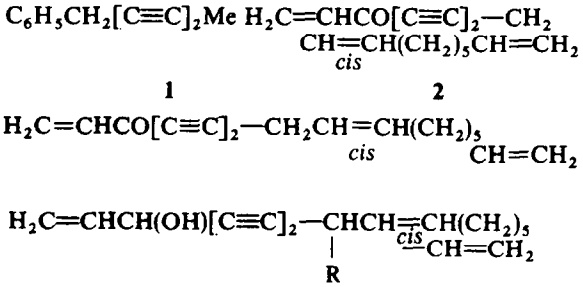
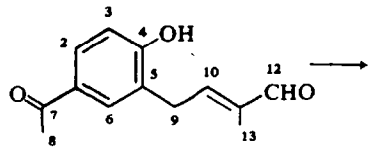
Key Word Index— *Artemisia monosperma*; Compositae; new *p*-hydroxyacetophenon-derivative.

Die Wurzeln der in Israel heimischen *Artemisia monosperma* enthalten die Acetylenverbindugen **1–4**, die auch in anderen Arten vorkommen [1]. Auch die oberirdischen Teile ergeben **1–4** sowie den Aldehyd **5**, dessen Struktur klar aus den spektroskopischen Daten und denendes durch Acetylierung erhaltenen Acetats (**6**) hervorgeht.



1 **2**

3, R = H **4**, R = OH



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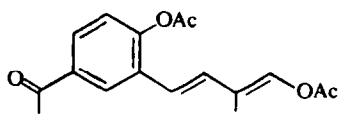

6

Wir möchten **5** Artemispermal nennen.

EXPERIMENTELLES

IR: Beckman IR 9 in CCl_4; ^{1}H-NMR: Bruker WH 270; MS: Varian MAT 711 mit Datenverarbeitung, 70 eV, Direkteinlaß. Die frisch zerkleinerten, in Israel gesammelten Pflanzenteile extrahierte man mit Ether und trennte die erhaltenen Extrakte zunächst grob durch SC (Si gel, GF 254). Als Laufmittel dienten Ether–Petrolether-Gemische (= E–P). 200 g Wurzeln ergaben 320 mg **1**, 60 mg **2**, 200 mg **3**, 46 mg **4**, während 300 g oberirdische Teile 90 mg **1**, 1 mg **2**, 25 mg **3**, 5 mg **4** und 16 mg **5** (E–P 3:1) lieferten.

Artemispermal (**5**). Farbloses Öl, IR: OH 3600; >C=O 1675; CHO 2730, 1675; C=C 1645; Aromat 1600 cm^{-1}. MS: M^+ *m/e* 218.094 (90%) (ber. für $C_{13}H_{14}O_3$ 218.094); $-CH_3$ 203 (39); 203 − CO 175 (44); 175 − CH_2O 145 (100). 10 mg **5** erwärmte man in 1 ml Acetanhydrid 1 h auf 70°. Nach Abdampfen des Anhydrids erhielt man nach DC (E–P 3:1) 7 mg **6**, farbloses Öl, IR: OAc 1755, 1240; >C=O 1658 cm^{-1}. MS: M^+ *m/e* 302.115

Tabelle 1. H-NMR-Daten von **5** und **6** (δ-Werte in ppm, 270 MHz, TMS als innerer Standard, $CDCl_3$)

	5	*J* (Hz)	**6**	*J* (Hz)
2-H	*dd* 7.79	2,3 = 8	*dd* 7.84	2,3 = 9
3-H	*d* 6.92	2,6 = 2	*d* 7.16	2,6 = 2
6-H	*d* 7.80		*d* 8.17	
8-H	*s* 2.57		*s* 2.62	
9-H	*d(br)* 3.73	9,10 = 7.2	*d* 6.87	9,10 = 16
10-H	*tq* 6.68	10,13 = 1	*d* 6.56	10,13 = 1
12-H	*s* 9.41		*s(br)* 7.46	
13-H	*d* 1.89		*d* 1.93	
OAc	—		*s* 2.23	
			s 2.38	

Obwohl *p*-Hydroxyacetophenon-Derivate bei Compositen sehr verbreitet sind [2], hat man deratige Substanzen aus Vertretern der Tribus *Anthemideae* noch nicht isoliert.