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24-METHYLENE-25-METHYLCHOLESTEROL, A STEROL FROM THE SEEDS OF *BRASSICA JUNCEA*

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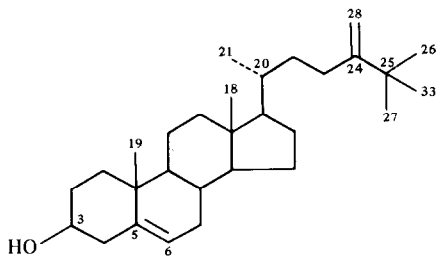
Key Word Index—*Brassica juncea*; Cruciferae; sterol; 24-methylene-25-methylcholesterol; seeds.

Abstract—A new sterol isolated from the seeds of *Brassica juncea* has been shown to be 24-methylene-25-methylcholesterol.

We have recently studied the 24-methyl-$\Delta^{5,22}$-sterol fractions, which were isolated from the seed oils of some *Brassica* and *Raphanus* species of Cruciferae plants, and demonstrated that the sterol fractions contained 10–40% of 24α-methylcholesta-5,*E*-22-dien-3β-ol in addition to its 24β-stereoisomer, 24β-methylcholesta-5,*E*-22-dien-3β-ol (brassicasterol) [1–3]. Our continuing study of the sterols of *B. juncea* seeds has now led to the isolation and characterization of a new sterol with an unusual side chain, 24-methylene-25-methylcholesterol [**1**, 24,25-dimethylcholesta-5,24(28)-dien-3β-ol].

The sterol fraction that was separated from the unsaponifiable lipid of *B. juncea* seed oil was acetylated and the resulting acetate fraction (1.8 g) was separated into four bands by silver nitrate–silica gel TLC. The fraction (44 mg) recovered from the most polar band (R_f 0.12) was subjected to reverse-phase HPLC which yielded a steryl (**1**) acetate (9 mg). GC and argentic TLC had shown that this sterol comprised 0.7% of the total sterols. The mass spectrum of **1**-acetate showed fragments at *m/z* 394 ($C_{29}H_{46}^+$, the ion of highest mass corresponding to loss of acetic acid from the molecular ion) and *m/z* 253 ($C_{19}H_{25}^+$, loss of side chain and acetic acid with 2H transfer) indicating that it was an acetate of a C_{29}-sterol with two double bonds, one of which was in the C_{10} side chain and the other probably located at C-5 [4, 5]. The side chain double bond was located either at the $\Delta^{24(25)}$- or $\Delta^{24(28)}$-position by the presence of the diagnostically significant ion of *m/z* 296 (base peak) due to a McLafferty rearrangement [4–6] involving cleavage of the C-22, C-23 bond with one H transfer from C-20 and loss of acetic acid. The 400 MHz ^{1}H NMR spectrum ($CDCl_3$) of **1**-acetate showed the following side chain signals: δ 0.965 (3H, *d*, *J* = 6.5 Hz), 1.058 (9H, *s*), 4.661 (1H, *s*) and 4.832 (1H, *s*) besides signals arising from the conventional Δ^5-3β-acetoxy sterol nucleus [7, 8] [δ 0.689 (3H, *s*, H-18), 1.022 (3H, *s*, H-19), 2.035 (3H, *s*, 3β-OAc), 4.60 (1H, *m*, $W_{1/2}$ = 28 Hz, H-3α) and 5.38 (1 H, *m*, H-6)]. The two olefinic singlets at δ 4.661 and 4.832, together with the diagnostic IR absorption at ν_{max} 895 cm^{-1} [9], indicated that the side chain double bond at C-24 must be oriented to C-24 (28) as the terminal methylene group [8, 10]. The *t*-butyl signal deshielded to δ 1.058 showed the presence of an additional methyl group at C-25 which is linked to the double bond [10, 11]. The remaining methyl doublet (δ 0.965) was then ascribed to the C-21 methyl substituent. The 20*S*-configuration is unlikely since this stereochemistry shifts the C-21 signal to the higher-field [12]. Thus the new sterol has a structure 24-methylene-25-methylcholesterol (**1**).

Chromatographic and GC/MS results have shown that **1** also occurs as a minor sterol constituent in the seeds of



1

other Cruciferae, i.e. *Brassica campestris* var. *periviridis*, *B. oleracea* var. *acephala*, *B. oleracea* var. *italica* and *Raphanus sativus* var. *longipinnatus* and *Helianthus annuus* (Compositae) [T. Matsumoto, S. Asano and T. Itoh, unpublished observations]. It is worth noting that the 24-ethylidene analog of **1**, 25-methylfucosterol [24-ethyl-25-methylcholesta-5,*E*-24(28)-dien-3β-ol], has been isolated quite recently from the marine sponge *Pseudoaxinyssa* sp. obtained from the Australian Great Barrier Reef [10]. On the other hand, a higher plant *Quercus myrsinaefolia* (Fagaceae) is known to contain a tetracyclic triterpene alcohol possessing a side chain isomeric to that of **1**, namely 24,25-dimethyl-5α-lanosta-9(11),23-dien-3β-ol [11].

EXPERIMENTAL

Mps are uncorr. HPLC was carried out on a Partisil 5 ODS-2 column (Whatman, 10 mm i.d. × 25 cm; packed by Erma Optical Works, Tokyo) using a UV detector monitoring at 212 nm (mobile phase, MeOH–H_2O, 98:2). GC on OV-17 and OV-1 SCOT glass capillary columns were under the conditions already described [13]. RR_t on HPLC and GC were expressed relative to cholesteryl acetate. MS (70 eV) were taken with a direct inlet system. ^{1}H NMR spectra were determined in $CDCl_3$ or in C_6D_6 with TMS as internal standard. The seeds of *B. juncea* were courteously supplied from Sakai Spice Industry Co. (Saitama). Isolation of the sterol fraction from the seed material and our general techniques have been described previously [1].

24-Methylene-25-methylcholesteryl (**1**) *acetate*. Mp 148–149°. RR_t: 0.97 on HPLC, and 1.68 (OV-17) and 1.58 (OV-1) on GC. MS: *m/z* 394.3582 ($M^+ - HOAc$, 80%, $C_{29}H_{46}$ requires 394.3596), 379.3364 ($C_{28}H_{43}$, 18), 296.2469 ($C_{22}H_{32}$, 100), 281.2282 ($C_{21}H_{29}$, 30), 253.1943 ($C_{19}H_{25}$, 31), 228.1873 ($C_{17}H_{24}$, 15), 213.1639 ($C_{16}H_{21}$, 19), 211.1498 ($C_{16}H_{19}$, 12). ^{1}H NMR (400 MHz, C_6D_6): δ 0.649 (3H, *s*, H-18), 0.925 (3H, *s*, H-19), 1.022 (3H, *d*, J = 6.4 Hz, H-21), 1.114 (9H, *s*, H-26, H-27, H-33), 1.753 (3H, *s*, 3β-OAc), 4.85 (1H, *m*, $W_{1/2}$ = 28 Hz, H-3α), 5.36 (1H, *m*, H-6), 4.901 and 5.038 (each 1H and *s*, H-28). IR ν_{max}^{KBr} cm^{-1}: 810, 833, 845 (>C=CH–), 895 (>C=CH_2), 1245, 1730 (OAc).

24-Methylene-25-methylcholesterol (**1**). Hydrolysis of **1**-acetate afforded free sterol **1**, mp 158.5–160°. MS: *m/z* 412.3684 (M^+, 5%, $C_{29}H_{48}O$ requires 412.3702), 397.3451 ($C_{28}H_{45}O$, 11), 379.3352 ($C_{28}H_{43}$, 5), 314.2569 ($C_{22}H_{34}O$, 100), 299.2349 ($C_{21}H_{31}O$, 24), 296.2495 ($C_{22}H_{32}$, 12), 281.2289 ($C_{21}H_{29}$, 28), 271.2056 ($C_{19}H_{27}O$, 29), 255.2095 ($C_{19}H_{27}$, 7), 253.1936 ($C_{19}H_{25}$, 10), 231.1732 ($C_{16}H_{23}O$, 10), 229.1961 ($C_{17}H_{25}$, 14), 213.1647 ($C_{16}H_{21}$, 17), 211.1472 ($C_{16}H_{19}$, 12). ^{1}H NMR (100 MHz, $CDCl_3$): δ 0.69 (3H, *s*, H-18), 1.01 (3H, *s*, H-19), 0.96 (3H, *d*, J = 6.5 Hz, H-21), 1.05 (9H, *s*, H-26, H-27, H-33), 3.53 (1H, *m*, $W_{1/2}$ = 28 Hz, H-3α), 5.38 (1H, *m*, H-6), 4.65 and 4.82 (each 1H and *s*, H-28).

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