ride on their parent carbon atoms. The orientations of all the methyl groups were determined from a ΔF map, and the groups refined as rigid bodies. It was not possible to determine the absolute configuration from the data. Refinement converged to give $R = 0.026$, $R_w = 0.028$, $[W^{-1} = \sigma^2(F) + 0.00025\,F^2]$. Maximum residual electron density was 0.05 eÅ^{-3} and maximum shift/error in final refinement were 0.02 and 0.06, respectively.

There is an intermolecular O–H . . . O (2.93 Å, OHO = 157°) between the hydroxyl oxygen, on C-10 and the methyl ester carbonyl oxygen.

Tables of final atom coordinates, temperature factors, and observed and calculated structure factors have been deposited at the Cambridge Crystallographic Data Centre.

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24-METHYLENE-25-METHYLLATHOSTEROL: A STEROL FROM *SICYOS ANGULATUS*

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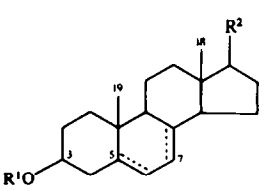
Key Word Index—*Sicyos angulatus*; Cucurbitaceae; sterol; 24-methylene-25-methyllathosterol.

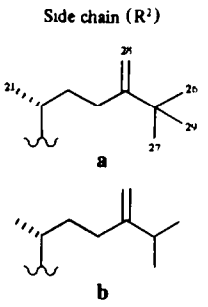
Abstract—A new sterol isolated from the aerial parts of *Sicyos angulatus* has been shown to be 24-methylene-25-methyllathosterol.

We have recently demonstrated the co-occurrence of C-24 epimers of some 24-ethyl-Δ^5- and Δ^7-sterols [1–4], and moreover of 24-ethyl-Δ^8-sterols [4], and 24-methylsterols [4], in plants of the family Cucurbitaceae. Our continuing study on the sterol constituents of cucurbitaceous plants has led to the isolation of a sterol from the aerial parts of *Sicyos angulatus* (bur cucumber), and this paper describes the characterization of the sterol as 24-methylene-25-methyllathosterol (24-methylene-25-methyl-5α-cholest-7-en-3β-ol or 25-methyl-5α-ergosta-7,24(28)-dien-3β-ol, **1a**) which is considered to be a new sterol.

The aerial parts (leaves and stems) of *S. angulatus* (29 kg) were air dried and the lipid (95 g) was extracted with CH_2Cl_2 in a Soxhlet extractor. The unsaponifiable lipid (42.5 g), obtained from the extracted lipid through alkaline hydrolysis (5% KOH in MeOH) under reflux followed by extraction with isopropyl ether, was subjected to column chromatography on silica gel (330 g) (hexane–Et_2O, hexane–EtOAc, and then MeOH as eluant) which provided a sterol fraction (2.6 g, $R_f = 0.19$ on analytical TLC). A portion of the sterol fraction was acetylated, and the steryl acetate (1.1 g) was subjected to argentation TLC to give six bands. The fraction (47 mg) recovered from the fifth band from the solvent front ($R_f = 0.18$ on argentation TLC), consisted of two major components with RR_t 1.98 (**2a**) and 1.61 (**2b**) on GC. This was then subjected to reverse-phase HPLC yielding **2a** (4 mg) and **2b** (4 mg) of which the latter was identified as 24-methylenelathosteryl (24-methylene-5α-cholest-7-en-3β-yl) acetate (**2b**).



1 5α-H, Δ^7, R¹ = H
2 5α-H, Δ^7, R¹ = Ac
3 Δ^5, R¹ = H
4 Δ^5, R¹ = Ac



The mass spectrum of **2a** showed a M^+ at *m/z* 454, corresponding to $C_{31}H_{50}O_2$, accompanied with fragmentation ions at *m/z* 379 ($C_{28}H_{43}^+$, loss of acetic acid and a methyl group) and 313 ($C_{21}H_{29}O_2^+$, loss of side chain 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 was in the skeleton [5, 6]. The presence of a further significant ion at *m/z* 356 ($C_{24}H_{36}O_2^+$), which was observed also for **2b**, due to a McLafferty rearrangement involving cleavage of the C-22, C-23 bond with one H transfer from C-20, suggested that the side chain double bond was located either at the $\Delta^{24(25)}$- or $\Delta^{24(28)}$-position [5–7]. The proton signals observed in the ^{1}H NMR spectrum of **2a** at δ0.544 (*s*, 18-H_3), 0.812 (*s*, 19-H_3), 2.030 (*s*, 3β-OAc), 4.70 (*m*, 3α-H), and 5.15 (*m*, 7-H), were consistent with the corresponding signals for **2b** (Table 1) and, hence, **2a** was regarded to possess a Δ^7-3β-acetoxy-5α-sterol skeleton. The side chain ^{1}H signals of **2a** were observed at δ0.970 (3H, *d*, *J* = 6.5 Hz), 1.059 (9H, *s*), 4.664 (1H, *s*), and 4.837 (1H, *s*), among which the two olefinic singlets, together with the diagnostic IR absorption at ν_{max} 890 cm^{-1} (C=CH_2), 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 singlet deshielded to δ1.059 suggested the presence of an additional methyl group at C-25 which is linked to the double bond [9–11]. The olefinic and *t*-butyl singlets as well as the remaining methyl doublet (δ0.970), which was attributed to the 21-H_3, were very similar to the corresponding side chain ^{1}H signals of 24-methylene-25-methylcholesteryl (24-methylene-25-methylcholest-5-en-3β-yl) acetate (**4a**) (Table 1) [9] and, therefore, the 24-methylene-25-methyl structure was assigned to the side chain of **2a**. The 20*S*-configuration is unlikely since this stereochemistry shifts the 21-H_3 signal to the higher field [12]. Thus, **2a** has the structure of 24-methylene-25-methyllathosteryl acetate. Hydrolysis of **2a** afforded a free sterol, 24-methylene-25-methyllathosterol (**1a**, $C_{29}H_{48}O_1$).

Tentative identification of the other sterols and determination of the composition of *S. angulatus* sterols were performed on the basis of argentation TLC [4] and GC [4, 13] data as the acetyl derivatives as follows: **2a** (2.8%), **2b** (2.0%), 24-methyl-22*E*-dehydrolathosterol (*RR*$_t$ of the acetyl derivative in GC, 1.36; 1.0%), 24-methyllathosterol (1.55; 1.2%), 24-ethylcholesterol (1.63; 1.3%), 24-ethyl-25-dehydrocholesterol (1.64; 1.7%), 24-ethyl-22*E*-dehydrolathosterol (1.70; 58.7%), 24-ethyl-22*E*,25-bisdehydrolathosterol (1.80; 2.8%), 24*Z*-ethylidenecholesterol (1.81; 0.7%), 24-ethyllathosterol (1.94; 13.4%), 24-ethyl-25-dehydrolathosterol (1.95; 1.5%), 24*E*-ethylidenelathosterol (2.04; 1.4%), 24*Z*-ethylidenelathosterol (2.15; 9.3%), and 24-ethyl-24(25)-dehydrolathosterol (2.31; trace).

Sterol **1a**, isolated from *S. angulatus*, is considered to be a new natural product, and this study seems to be the second instance of the detection of the sterol possessing a 24-methylene-25-methylated side chain. The Δ^5-isomer of **1a**, i.e. 24-methylene-25-methylcholesterol (**3a**), has previously been shown to occur in the seeds of *Brassica juncea* and some other Cruciferae plants [9].

EXPERIMENTAL

Mps are uncorr. Analytical TLC on a pre-coated silica gel was developed once with hexane–EtOAc (6:1). Argentation TLC (silica gel–$AgNO_3$, 4:1) was developed four times with CCl_4–CH_2Cl_2 (5:1). HPLC was carried out on a Partisil 5 ODS-2 column (Whatman; 25 cm × 10 mm i.d.) with MeOH as a mobile phase (flow rate, 4 ml/min) which was monitored by an RI detector. GC on OV-17 SCOT glass capillary column (30 m × 0.3 mm i.d., column temp. 260°) were under the conditions already described [13]. *RR*$_t$ on HPLC and GC were expressed relative to cholesteryl acetate. The IR spectrum was taken in KBr. EI-MS (70 eV) were recorded by means of a probe injection. ^{1}H NMR spectra (400 MHz) were determined in $CDCl_3$ with TMS as internal standard. Acetylation was performed in Ac_2O–pyridine room temp. overnight. The aerial parts of *Sicyos angulatus* were collected at the bank of Asakawa River (Hino-shi, Tokyo) in August, 1985. The origin of 24-methylene-25-methylcholesterol (**3a**) [9], 24-methylenelathosterol (**1b**) and the other authentic sterols [4], used for the identification of *S. angulatus* sterols, was described previously. For the ^{1}H NMR data of **1a**, **2a**, **2b**, and **4a**, see Table 1.

24-Methylene-25-methyllathosterol (**1a**). Mp 167–168°. MS: *m/z* 412.3675 (M^+, $C_{29}H_{48}O_1$, rel. int. 9%, requires 412.3702), 397.3514 ($C_{28}H_{45}O_1$, 14%), 314.2577 ($C_{22}H_{34}O_1$, 41%), 299.2346 ($C_{21}H_{31}O_1$, 9%), 285.2235 ($C_{20}H_{29}O_1$, 5%), 271.2037

Table 1. ^{1}H NMR data (400 MHz; $CDCl_3$)* of some 24-methylenesterols

Sterol	18-H_3 (*s*)	19-H_3 (*s*)	21-H_3 (*d*)	26-H_3/27-H_3/ 29-H_3	28-H_2 (each 1H)	3β-OAc (*s*)	3α-H (*m*)	6-H or 7-H (*m*)
1a	0.545	0.799	0.969 (6.4)	1.058 (*s*)	4.663 (*s*) 4.835 (*s*)	—	3.60 (25)	5.16 (11)
2a	0.544	0.812	0.970 (6.8)	1.059 (*s*)	4.664 (*s*) 4.837 (*s*)	2.030	4.70 (25)	5.15 (11)
2b†	0.538	0.811	0.954 (6.7)	1.025 (3H, *d*, 6.7)‡ 1.030 (3H, *d*, 6.7)‡	4.659 (*d*, 1.3) 4.715 (*s*)	2.029	4.70 (25)	5.15 (11)
4a	0.689	1.022	0.965 (6.5)	1.058 (*s*)	4.661 (*s*) 4.832 (*s*)	2.035	4.60 (28)	5.38 (9)

*Chemical shifts given in δ values from TMS; figures in parentheses denote *J* values (Hz) for doublet signals, otherwise $W_{1/2}$ values (Hz) for multiplet signals; *s* = singlet, *d* = doublet, *m* = multiplet.

†Other signal: δ2.232 (1H, septet, *J* = 6.7 Hz, 25-H).

‡26-H_3 and 27-H_3 signals.

($C_{19}H_{27}O_1$, 100%), 255.2105 ($C_{19}H_{27}$, 13%), 246.1937 ($C_{17}H_{26}O_1$, 11%), 231.1790 ($C_{16}H_{23}O_1$, 13%), 227.1807 ($C_{17}H_{23}$, 10%), 213.1658 ($C_{16}H_{21}$, 11%).

24-Methylene-25-methyllathosteryl acetate (**2a**). Mp 145–146°. RR_t: 0.85 on HPLC, and 1.98 on GC. IR ν_{max} cm^{-1}: 1735, 1245 (OAc), 890 (C=CH_2), 822, 795 (C=CH). MS: *m/z* 454.3853 (M^+, $C_{31}H_{50}O_2$, rel. int. 9%, requires 454.3808), 439.3614 ($C_{30}H_{47}O_2$, 12%), 379.3402 ($C_{28}H_{43}$, 4%), 356.2718 ($C_{24}H_{36}O_2$, 43%), 342.2596 ($C_{23}H_{34}O_2$, 9%), 313.2130 ($C_{21}H_{29}O_2$, 100%), 296.2517 ($C_{22}H_{32}$, 4%), 288.2112 ($C_{19}H_{28}O_2$, 6%), 281.2295 ($C_{21}H_{29}$, 4%), 273.1853 ($C_{18}H_{25}O_2$, 6%), 255.2101 ($C_{19}H_{27}$, 14%), 253.1969 ($C_{19}H_{25}$, 6%), 227.1844 ($C_{17}H_{23}$, 14%), 213.1638 ($C_{16}H_{21}$, 20%).

24-Methylenelathosteryl acetate (**2b**). Mp 149–150°. RR_t: 0.80 on HPLC, and 1.61 on GC. MS: *m/z* 440 (M^+, rel. int. 16%), 425 (14%), 380 (5%), 365 (6%), 356 (39%), 342 (9%), 341 (5%), 313 (100%), 288 (5%), 281 (5%), 273 (8%), 255 (25%), 253 (11%), 227 (16%), 213 (27%).

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