82 mg of pure product: mp 178–182°; $[\alpha]_D$ +74°; ν_{max} (KBr) 3620 cm^{-1}.

Anal. Calcd for $C_{30}H_{50}O$: C, 84.44; H, 11.81. Found: C, 84.42; H, 11.75.

6-Oxoolean-12-ene (1g).—Daturadione **1h** (95 mg) was reduced in the same manner to give 69 mg of the product: mp 174–176°; $[\alpha]_D$ +88°; ν_{max} (KBr) 1705 cm^{-1}.

Anal. Calcd for $C_{30}H_{48}O$: C, 84.84; H, 11.4. Found: C, 84.74; H, 11.60.

Oxidation of the alcohol **1f** with Jones reagent yielded the same ketone, as proved by ir and mixture melting point.

3-Oxoolean-5,12-diene (4).—Daturaolone (205 mg) in 5 ml of pyridine was treated with 1 ml of thionyl chloride in an ice bath. The usual work-up and crystallization from dilute alcohol yielded 173 mg of pure product: mp 169–171°; ν_{max} (KBr) 1700, 1660, 840 cm^{-1}.

Anal. Calcd for $C_{30}H_{46}O$: C, 85.24; H, 11.0. Found: C, 85.16; H, 11.15.

Registry No.—**1a**, 41498-79-7; **1b**, 41498-80-0; **1c**, 41579-25-3; **1d**, 41498-81-1; **1e**, 41498-82-2; **1f**, 41498-83-3; **1g**, 41498-84-4; 5α-**1h**, 41498-85-5; 5β-**1h**, 41498-86-6; **1h**-d_5, 41499-07-4; **2**, 41498-87-7; **3**, 41498-88-8; **4**, 41498-89-9.

Cyclotrichosantol, a New C_{31} 31-Nortriterpene

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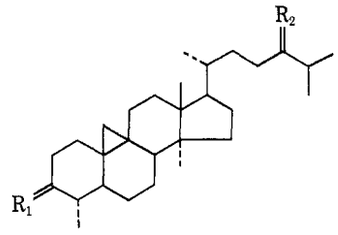
Isolation of cycloeucalenol **1a** and a novel methylsterol—cyclotrichosantol (**2a**, 4α,14α-dimethyl-24-ethyl-9:19-cyclocholest-25-en-3β-ol)—from the leaves of *Trichosantes palmata* L., Cucurbitaceae, is described. The structure was established on the basis of mass and nmr spectra with the use of $Eu(dpm)_3$. The probably biogenetic significance of cyclotrichosantol is discussed.

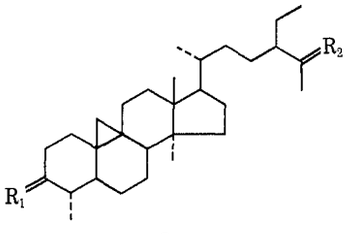
Although the biogenesis of C-29 plant sterols containing a 24-ethyl group by way of the precursors (24-methylenecycloartenol, cycloeucalenol, obtusifiol, and citrostadienol) has been well established,[1] it seems probable that this is not the only possible biogenetic pathway. It is also possible that certain minor Δ^{25} sterols, widely distributed in some plants,[2a] especially in those of the Cucurbitaceae family,[2b,c] are markers of an alternative pathway for the introduction of a 24-ethyl group, just as similarly 24-ethylidenesterols, *e.g.*, Δ^5 and Δ^7 avenasterols, are considered to be markers of such a pathway.

In the present paper we describe the structure elucidation of a new cyclopropane methylsterol, cyclotrichosantol **2a**, which was isolated from the leaves of *Trichosantes palmata* L. (Cucurbitaceae), an Indian medicinal plant, together with cycloeucalenol **1a**.

The so-called "methylsterol" fraction was isolated from the saponified petroleum ether (bp 30–60°) extract of the dry leaves by chromatography and crystallization subsequent to treatment of the whole saponified extract with urea to remove the aliphatic alcohols which mask the minute methylsterol fraction. Only two components were present as shown by tlc, and they were separated by preparative tlc, the less polar in 0.005 and the more polar in 0.009% yield based on the weight of dry leaves. The compound with lower R_f value was easily identified as cycloeucalenol **1a** on the basis of its properties (melting point, ir, mass spectrum, and pmr). This was confirmed by preparation of the acetate **1b**, the 3 ketone **1c**, and the 28 norketone **1d**, the properties of which were in good agreement with reported data.[3]



1



2

	R_1	R_2
a	β-OH, α-H	$=CH_2$
b	β-OAc, α-H	$=CH_2$
c	$=O$	$=CH_2$
d	β-OH, α-H	$=O$

The second compound—cyclotrichosantol—like cycloeucalenol **1a** exhibited the presence of cyclopropane and methylene groups in its ir and pmr spectra. Both compounds melted at nearly the same point (for cycloeucalenol mp 144–145°, for cyclotrichosantol mp 143–144°), and practically no depression was noted for the mixture, a circumstance which demonstrated the close relationship of the two compounds. Cyclotrichosantol gave an acetate **2b**, a 3 ketone **2c**, and a 26 norketone **2d**, the latter by oxidation with OsO_4–KIO_4.

Molecular ions in the mass spectra of all these compounds corresponded to the formula $C_{31}H_{52}O$ for the parent alcohol. The two previously known triterpenes having this composition, 24-methylenecycloartenol and cyclolaudenol, were excluded, as both melt at distinctly lower temperature (122 and 125°, respectively[4]).

Structure **2a** for cyclotrichosantol could, however,

(1) L. J. Goad in "Natural Substances Formed Biologically from Mevalonic Acid," T. W. Goodwin, Ed., Academic Press, New York, N. Y., 1970.

(2) (a) W. Sucrow, *Chem. Ber.*, **99**, 3559 (1966); M. Manzoor-i-Khuda, *Tetrahedron*, **22**, 2377 (1966); T. Sedane and T. Villacorta, *An. Real. Soc. Espan. Fis. Quim. Ser. B*, **66**, 1315 (1970); L. M. Belger, N. N. Rees, E. L. Ghisalberti, L. J. Goad, and T. W. Goodwin, *Tetrahedron Lett.*, 3043 (1970); S. C. Pakrashi and B. Achari, *ibid.*, 365 (1971). (b) R. R. Gonzales and F. M. Panizo, *An. Real. Soc. Espan. Fis. Quim. Ser. B*, **63**, 1123 (1967); W. Sucrow and A. Reimerdes, *Z. Naturforsch. B*, **23**, 42 (1968). (c) I. Belič, T. Čerin, and D. Stucin, *Vestn. Slov. Kem. Drûs.*, **18**, 1 (1971).

(3) J. S. C. Cox, F. F. King, and T. J. King, *J. Chem. Soc.*, 514 (1959).

(4) "Rodd's Chemistry of Carbon Compounds," Vol. IIC, 1969, p 423.

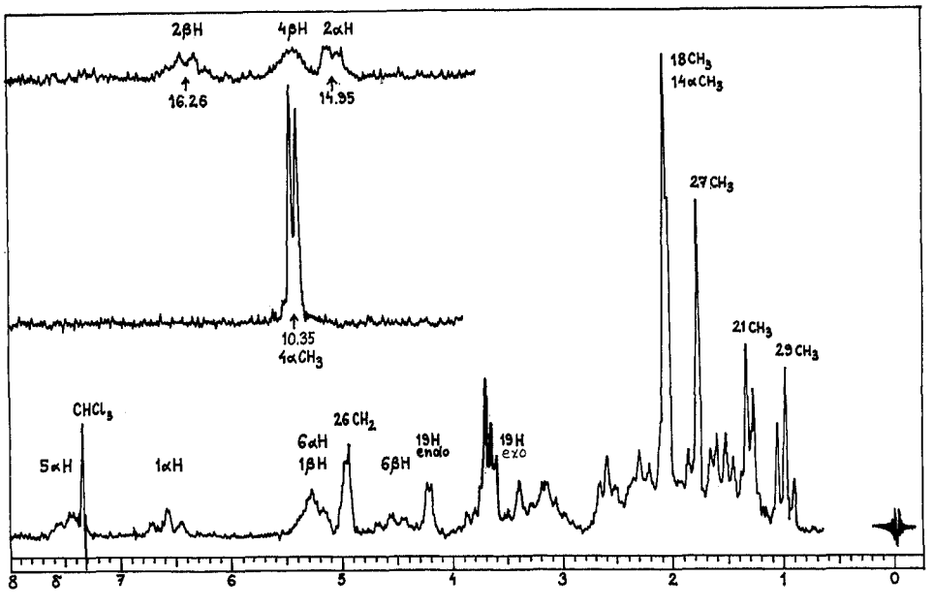


Figure 1.—100-MHz pmr spectrum of cyclotrichosantol (**2a**) in $CDCl_3$ with added $Eu(dpm)_3$; concentration ratio 1.02.

be deduced by comparing the mass spectra of **2a**, **2b**, **2c**, and **2d**.

Mass spectra of **2a**, **2b**, and the norketone **2d** showed a peak corresponding to the elimination of 55 mass units from the ion M − 18 (or M − 60); this process is also observed in the case of **1a**, **1b**, and **1d**, and is typical for 4-methyl-9:19-cyclosterols.[5] The fragment 55 represents carbon atoms 2, 3, and 4 and 4-Me. The second characteristic process involving elimination of ring A, for which two alternative mechanisms have been proposed,[5,6] was also visible in the spectra: M − 126 in **2a** and **2d**, also in **1a** and **1d**, and M − 168 in **2b** and **1b**. In the 3 ketones the analogous elimination of *m/e* 124 was observed. These facts are consistent with the presence of one methyl group at C-4 of cyclotrichosantol, as in the case of cycloeucalenol.

Cyclotrichosantone and cycloeucalenone (**2c** and **1c**) exhibited nearly the same value of $\Delta\epsilon_{285}$ in their CD spectra (1.08 and 1.11, respectively). This value is typical for 4α-methyl-3-ones.[7]

The presence of the $C_{10}H_{19}$ side chain was evident from the appearance of the ion M − 139 in the spectra of **2a–c** which was displaced to M − 141 in the spectrum of **2d**. Because the ion M − 139 − 2 was very weak, the side chain unsaturation should be distant from the nucleus.[8] The elimination of part of the side chain in a McLafferty process was also visible, especially in the spectrum of **2c** (M − 84), and the corresponding elimination of *m/e* 86 was present in the spectrum of **2d**. The pmr spectrum of **2a** or its acetate **2b** showed the presence of a methyl group connected with the double bond (broadened singlet at δ 1.60). Therefore any other arrangement of the side chain was excluded.

The use of the $Eu(dpm)_3$ shift reagent provided further confirmation for the ring skeletal identity of **1a** and **2a** and revealed the presence of an ethyl group whose signal was invisible in the normal pmr spectrum of **2a** (Figure 1).

That the carbocyclic skeletons of **1a** and **2a** are identical is clearly seen by inspection of Table I, which shows that the shifts of the methyl groups in the two compounds are nearly the same; furthermore at high Eu-complex concentration all ring A protons signals were visible, and these parts of both spectra were nearly identical. The spectrum of cyclotrichosantol was decoupled for all axial–axial and geminal couplings; the most shifted methyl group signal, the doublet of 4α-Me, which is absent from the spectra of similar 4,4-dimethyl derivatives,[9] is coupled to the broad signal at 15.32 ppm which must therefore be the resonance of 4α-H. The remaining signals could then be identified as shown in Figure 1.

This example demonstrates the utility of shift reagents for a comparison of closely related compounds which differ from each other at locations far from the complexing group, a situation which is encountered frequently in sterols and terpenes.[10,11]

Cyclotrichosantol **2a** thus possesses a ten-carbon-atom side chain, like all "finished" plant sterols. In comparison with the initial cyclic plant sterol precursor, cycloartenol, **2a** lacks only one methyl group, namely, the one at C-4 (if the presence of the "extra" ethyl group at C-24 is neglected). Therefore, if cyclotrichosantol **2a** is not a side product of sterol biosynthesis in *Trichosantes palmata*, but a sterol precursor, the hypothetical "25-methylene" path differs from the "24-ethylidene" path not only in the manner of 24-alkylation, but also in the order of skeletal demethylation and double bond formation.

Recently another methylsterol was isolated from *Echinocystis lobata*, a member of the Cucurbitaceae, and the structure 4α-methylstigmasta-7,25-dien-3β-ol was proposed for it as the most likely possibility.[2c] This methylsterol thus represents the 25(26) isomer of

(5) H. E. Audier and B. C. Das, *Tetrahedron Lett.*, 4341 (1966).
(6) R. T. Aplin and G. M. Hornby, *J. Chem. Soc. B*, 1078 (1966).
(7) L. H. Zalkow, R. Hale, K. French, and P. Crabbé, *Tetrahedron*, **26**, 4948 (1970).
(8) S. C. Wyllie and C. Djerassi, *J. Org. Chem.*, **33**, 305 (1968).
(9) J. St. Pyrek, unpublished work.
(10) O. Achmatowicz, Jr., A. Ejchart, J. Jurczak, L. Kozerski, and J. St. Pyrek, *Chem. Commun.*, 98 (1971).
(11) Unpublished work.

TABLE I

CHEMICAL SHIFTS, δ, AND RELATIVE PARAMAGNETIC SHIFTS, ΔEu, CAUSED BY Eu(dpm)$_3$ IN $CDCl_3$[a]

	Cyclotrichosantol (2a)		Cycloeucalenol (1a)		
	δ	ΔEu		δ	ΔEu
30-Me (4α-Me)	d 1.00	100	30-Me (4α-Me)	d 1.00	100
18-Me (13β-Me)	s 1.01	11:9	18-Me (13β-Me)	s 1.01	11.6
32-Me (14α-Me)	s 0.92	12.2	32-Me (14α-Me)	s 0.94	12.0
21-Me	d 0.88	4.6	21-Me	d 0.94	4.5
27-Me	b s 1.60	2.0	26,27-Me_2	d 1.08	1.6
26-CH_2 (25 =CH_2)	4.78	2.4	28-CH_2 (24 =CH_2)	4.73	2.5
	4.71			4.77	
29-Me	t 0.81	1.8			
3α-H	b 3.23	225	3α-H		213

[a] Shifts are normalized assuming a 4α-Me shift value of 100.

citrostadienol and should be regarded as the last sterol precursor in the proposed biosynthetic scheme. A methylsterol with the same skeleton as cyclotrichosantol, but with a methyl group at C-24, *i.e.*, a nine-carbon side chain, 31-norcyclolaudenol, has been isolated from the rhizomes of *Polypodium vulgare* L.,[12] and the corresponding ketone has been found in *Musa sapientum* L.[13] Cycloneolitsin and cyclolabanone, two "abnormal" 24-methylation products, should be mentioned also; these C_{32} triterpenes possess two geminal methyl groups at C-24 and a 25(26) double bond.[14]

The sterol fraction of *Trichosantes palmata* L. leaves were examined also. The main sterol component was identified as α-spinasterol, but by tlc chromatography and mass spectrometry trace amounts of stigmastanol and C-29 sterols with 7 or 7,25 or 7,22,25 double bonds were shown to be present also.

Experimental Section

Isolation of Methylsterols.—The petroleum ether extract, 48 g, of dry *Trichosantes palmata* L. leaves, 1.5 kg, was saponified with 20 g of NaOH in 50 ml of water, 400 ml of ethanol, and 100 ml of benzene under a nitrogen atmosphere at reflux temperature for 4 hr. The mixture was acidified with acetic acid, diluted with 500 ml of water, and extracted with ether. The residue from the ether extract was dissolved in 200 ml of ethyl acetate and 400 ml of methanol, and 100 g of urea was added with boiling. The clathrates were separated and the solvent was again removed. The residue was extracted with ether and washed with water and alkali; evaporation gave 32.5 g of oily residue which was chromatographed on 550 g of alumina grade IV–V with petroleum ether as solvent. The methylsterol fraction was crystallized from hexane and chromatographed on silica gel plates containing 30% of $AgNO_3$ with benzene as developing solvent.

Cycloeucalenol (1a).—1a (130 mg) was obtained after crystallization from dilute alcohol: mp 144–145° (lit.[3] mp 138–139°); ν_{max} (KBr) 3400, 1640, 885 cm^{-1}; nmr (100 MHz, $CDCl_3$) δ 4.77 (1 H, b s), 4.73 (1, H, b s), 3.25 (1 H, b), 1.08 (6 H, d, J = 6 Hz), 1.01 (3 H, s), 0.94 (3 H, d, J = 6 Hz), 0.94 (3 H, s), 0.1–0.4 (2 H, q, J = 4 Hz); mass spectrum m/e (rel intensity) 426 (45), 411 (58), 408 (100), 393 (70), 383 (10), 356 (6), 353 (9), 342 (6), 327 (10), 324 (7), 309 (10), 301 (30), 300 (40). Acetate **1b** had mp 105–109°; $[\alpha]_D$ +61.6° (c 0.66, $CHCl_3$) (lit.[3] mp 110°, $[\alpha]_D$ +63°); ν_{max} (KBr) 1730, 1640, 1243, 900 cm^{-1}; nmr δ (100 MHz, CCl_4) 4.75 (1 H, b s), 4.71 (1 H, b s), 4.43 (1 H, b), 2.00 (3 H, s); mass spectrum m/e (rel intensity) 468 (12), 453 (9), 425 (5), 408 (100), 393 (52), 365 (5), 353 (9), 325 (7), 324 (5), 300 (12), 283 (14), 281 (10).

Cycloeucalenone (1c).—Cycloeucalenol (**1a**) was oxidized with excess CrO_3 in pyridine and purified by preparative tlc and crystallization from methanol: mp 83–84° (lit.[3] mp 84°); $\Delta\epsilon_{285}$ (c 0.2, MeOH) +1.11; mass spectrum m/e (rel intensity) 409 (18), 424 (45), 381 (19), 341 (17), 340 (18), 328 (13), 327 (13), 326 (12), 325 (10), 300 (18), 299 (36).

28-Norcycloeucalenol (1d).—Cycloeucalenol (7 mg) was oxidized with excess KIO_4 in 2 ml of 80% acetic acid and in the presence of 2 mg of OsO_4. The more polar product was purified by preparative tlc and crystallization from dilute methanol: yield 5 mg; mp 114–116° (lit.[3] 110–111°); $\Delta\epsilon_{285}$ (c 0.2, MeOH) −0.185; mass spectrum m/e (rel intensity) 428 (48), 413 (50), 410 (100), 395 (80), 385 (4), 367 (6), 355 (18), 342 (7), 302 (47), 301 (27), 283 (27).

Cyclotrichosantol (2a).—Cyclotrichosantol (70 mg) was obtained after crystallization from dilute alcohol: mp 143–144°; $[\alpha]_D$ +42°; λ_{max} (EtOH) 195 nm (log ε 3.85); ν_{max} (KBr) 3450, 1640, 1040, 885 cm^{-1}; nmr (100 MHz, $CDCl_3$) δ 4.78 (1, H, b s), 4.71 (1 H, b s), 3.23 (1 H, b), 1.60 (3 H, b s), 1.01 (3 H, s), 0.92 (3 H, s), 0.88 (3 H, d, J = 6 Hz), 0.1–0.4 (2 H, q, J = 4 Hz); mass spectrum m/e (rel intensity) 440 (55), 425 (74), 422 (100), 407 (75), 367 (17), 314 (45), 301 (32). The acetate **2b** melted at 113°; $[\alpha]_D$ +58°; ν_{max} (KBr) 1735, 1640, 1250, 895 cm^{-1}; nmr (CCl_4, 100 MHz) δ 4.72 (1 H, b s), 4.64 (1 H, b s), 4.40 (1 H, b), 2.00 (3 H, s), 1.59 (3 H, s); mass spectrum m/e (rel intensity) 482 (17), 467 (11), 422 (100), 407 (61), 367 (10), 343 (7), 314 (13), 283 (23).

Cyclotrichosantone (2c).—Cyclotrichosantol (**2a**) was oxidized with excess CrO_3 in pyridine and purified by preparative tlc and crystallization from methanol: mp 121–122°; λ_{285} (c 0.15, MeOH) +1.08; mass spectrum m/e (rel intensity) 438 (51), 423 (20), 395 (5), 354 (12), 341 (11), 328 (18), 314 (12), 299 (69), ... 55 (100).

26-Norcyclotrichosantol (2d).—Cyclotrichosantol was oxidized as before with KIO_4–OsO_4 but a longer reaction time was required as compared with cycloeucalenol. The product was purified by preparative tlc and crystallization from dilute methanol: mp 125–127°; λ_{285} (c 0.1, MeOH) −0.07;[15] mass spectrum m/e (rel intensity) 442 (7), 427 (13), 424 (29), 409 (35), 369 (8), 356 (5), 316 (13), 301 (20), 283 (18), ... 55 (100).

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Registry No.—**1a**, 469-39-6; **1b**, 10376-42-8; **1c**, 1255-12-5; **1d**, 15371-62-7; **2a**, 41507-26-0; **2b**, 41507-27-1; **2c**, 41507-28-2; **2d**, 41507-29-3.

(12) G. Berti, F. Bottari, B. Macchia, A. Marsili, G. Ourisson, and K. Piotrowska, *Bull. Soc. Chim. Fr.*, 2359 (1964).

(13) F. F. Knapp and H. J. Nicholas, *Steroids*, **16**, 329 (1970).

(14) E. Ritchie, R. G. Senior, and W. C. Taylor, *Aust. J. Chem.*, **22**, 2371 (1969); Y. Tachi, S. Taga, Y. Kamano, and M. Komatsu, *Chem. Pharm. Bull.*, **19**, 2193 (1971).

(15) This value of $\Delta\epsilon$ cannot be used for determination of the absolute configuration at C-24.