Tirucalla-7,24-dienol: A New Triterpene Alcohol from Tea Seed Oil

T. ITOH, T. TAMURA, and **T. MATSUMOTO**
College of Science and Technology, Nihon University, 8, Kanda Surugadai, 1-chome, Chiyoda ku, Tokyo, 101 Japan

ABSTRACT

A new triterpene alcohol is isolated from tea (*Thea sinensis*, Theaceae) seed oil, and its structure is shown to be 5α-tirucalla-7,24-dien-3β-ol. This triterpene alcohol is considered to be a possible biogenetic precursor to the meliane and meliacan series of oxygenated triterpenes. Gas liquid chromatographic and proton magnetic resonance spectroscopic correlations between euphane and tirucallane series triterpenes also are discussed.

INTRODUCTION

In the previous study on the unsaponifiable fraction of tea (*Thea sinensis*) seed oil, the presence of several unidentified triterpene alcohols besides β-amyrin, butyrospermol, and lupeol was indicated in the triterpene alcohol fraction of this oil (1).

This paper describes a further study of one of these unidentified triterpenes leading to the conclusion that it is 5α-tirucalla-7,24-dien-3β-ol (Fig. 1, IIc), a new triterpene alcohol from natural sources.

EXPERIMENTAL PROCEDURES

Authentic specimens of 5α-eupha-8,24-dien-3β-ol (Fig. 1, Ia, euphol) and 5α-tirucalla-8,24-dien-3β-ol (Ic, tirucallol) were obtained as gifts, and 5α-eupha-7,24-dien-3β-ol(IIa, butyrospermol) was isolated from tea seed oil (1). Three triterpenes of 5α-lanostane series (2)–(24ξ)-24-methyl-5α-lanost-9(11)-en-3β-ol (IIIe), 5α-lanosta-8,24-dien-3β-ol (IVa, lanosterol), and 5α-lanost-7-en-3β-ol (Vb)—also are used as reference specimens.

Melting points were determined with a micro melting point apparatus (Yanagimoto Seisakusho Ltd., Kyoto) and are uncorrected. All recrystallizations were performed in acetone-methanol. Preparative argentation thin layer chromatography ($AgNO_3$-TLC) for the fractionation of triterpene acetates was carried out on 20 x 20 cm plates coated with 0.5 mm of silica gel (Wakogel B-10, Wako Pure Chemical Industries Ltd., Osaka) impregnated with 10%

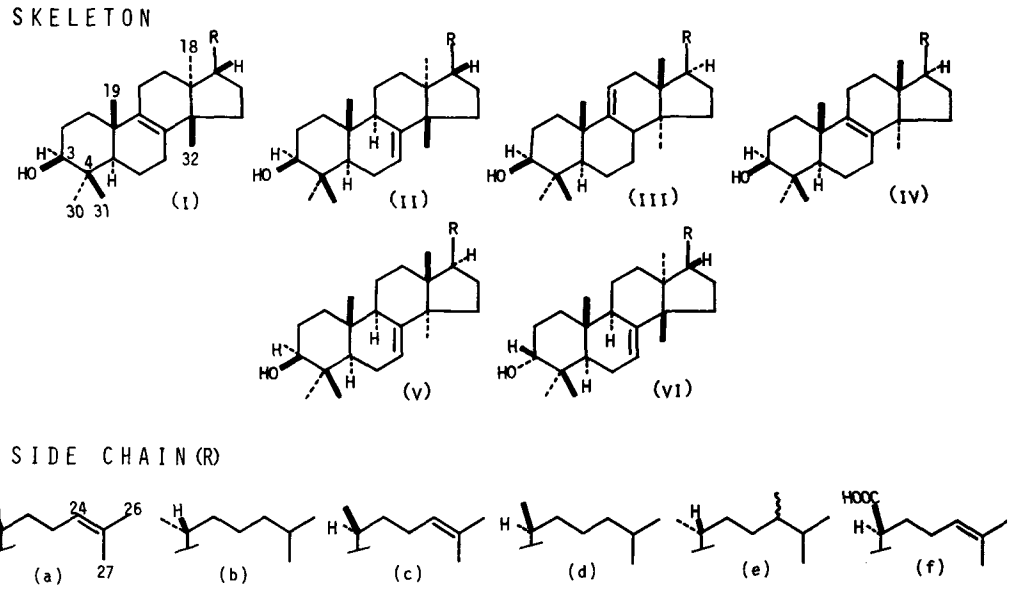

FIG. 1. Diagram of the skeletons (I-VI) and side chains (a-f) of euphane, tirucallane, and lanostane series of triterpenes.

or 20% silver nitrate. Methylene chloride was used as the eluting solvent. After development (17 cm), the separated zones were visualized and recovered as described previously (1). Analytical TLC of free triterpene alcohols was carried out on the 20 x 20 cm plate spread with 0.25 mm of silica gel. A mixture of hexane: ether:acetic acid (80:20:1) was used as the eluting solvent. Gas liquid chromatography (GLC) was performed on a Shimadzu GC-5A gas chromatograph (Shimadzu Seisakusho Ltd., Kyoto) equipped with a flame ionization detector and a 2 m x 3 mm inside diameter glass column packed with 3% OV-17 on Gas Chrom-Z, 80-100 mesh, prepared by Nihon Chromato Works Ltd. (Tokyo). The chromatograph was operated at a column temperature of 255 C. The carrier gas was nitrogen with a flow rate of 50 ml/min. Detector and injection port temperatures were 280 C. Relative retention time (RRT) was expressed by the ratio of the retention time to that of sitosterol (β-sitosterol, [24*R*]-24-ethylcholest-5-en-3β-ol). The figures indicated were the mean values determined on several runs.

Infrared (IR) spectra were recorded in KBr pellets on a Type IRA-2, IR spectrophotometer (Japan Spectroscopic Co., Tokyo). Optical rotations were measured in $CHCl_3$ using a Carl Zeiss Polarimeter 0.01° (Carl Zeiss, Oberkochen, West Germany). Concentrations used were indicated in parentheses as g/100 ml.

Proton magnetic resonance (PMR) spectra were recorded on a JNM-C-60-HL (60 MHz) or on a JNM-MH-100 (100 MHz) instrument (Japan Electron Optics Laboratory Co., Tokyo) for the solutions (0.2 M) of 80 μmol of triterpenes in 0.4 ml of deuteriochloroform ($CDCl_3$) at 28 C. The chemical shifts (δ) and the lanthanide-induced shifts (LIS) (δ[LSR]) were expressed in ppm downfield from internal tetramethylisilane. The LIS were recorded at 60 MHz with the solutions of triterpenes in the presence of a molar equivalent of tris (dipivalomethanato) europium (Eu[DPM]$_3$), a lanthanide shift reagent (LSR). For the convenience of comparison, the LIS were normalized as proposed by Buckley et al. (3). In this study, the δ(LSR) 11.00 was given to the lowest field methyl signal, 31 (4β)-methyl signal. The experimental details of this PMR work with the LSR were described in the previous paper (4).

Mass spectra were taken with a Hitachi RMU-7M mass spectrometer (Hitachi Ltd., Tokyo), electron energy 70 eV, target current 42 or 58 μA, ion source temperature 180 C, sample temperature 110-120 C, and accelerating high voltage 4.5 KV. The samples were introduced directly into the ion source through a vacuum-lock.

Other procedures such as hydrogenation (platinum oxide catalyst, ether solution), acetylation, and hydrolysis of triterpenes were carried out in a similar manner as described previously (1).

Isolation of an Unknown Triterpene Alcohol from the Unsaponifiable Material of Tea Seed Oil

A solution of unsaponifiable material (43.7 g) separated from tea seed oil (9.3 kg) in hexane was poured onto a column containing 1 kg of alumina (Wako). The following eluting solvents were then passed in succession: (i) 2.4 L (liters) of hexane (H), fraction 1, 10.3 g; (ii) 6.0 L of H-ether (E) (95:5), fraction 2, 9.2 g; (iii) 4.8 L of H-E (9:1) followed by 7.0 L of H-E (4:1), fraction 3, 1.2 g; (iv) 1.8 L of H-E (7:3), fraction 4, 2.8 g; (v) 7.0 L of H-E (7:3), fraction 5, 7.1 g; and (vi) 13.6 L of H-E (1:1), fraction 6, 1.7 g. The fractions 2-6 consisted exclusively of triterpene alcohols.

The fraction 5 (7.1 g) was acetylated and the product was recrystallized. The crystalline material consisting almost exclusively of the acetates of lupeol and butyrospermol was left aside from the further investigation. On the other hand, the filtrate was recovered to give a yellow, pasty solid (3.1 g), which was then roughly fractionated into five fractions by preparative $AgNO_3$-TLC. The fraction (1,600 mg) from the second zone from the starting line contained an unknown triterpene acetate and also the acetates of lupeol and butyrospermol. This fraction was further fractionated by preparative $AgNO_3$-TLC into seven fractions, of which the fraction (637 mg) from the third zone from the starting line eventually gave a uniform triterpene acetate (110 mg, >99% pure by GLC, RRT 1.59) after repeated recrystallization. The melting point was 123-124 C (needles), $[\alpha]_D^{21}$ -39° (c 0.82). The IR spectrum showed bands at 842 and 822 (trisubstituted double bond), 1730 and 1242 (acetoxyl), and 1394, 1380, and 1370 (geminal dimethyl) cm^{-1}. The overall patterns of the spectrum are closely similar to those recorded for eupha-7,24-dienyl (Fig. 1, IIa) acetate. The acetate on hydrolysis gave free alcohol (RRT 1.32), mp 76-79 C (fine needles). This unknown triterpene alcohol is shown by high resolution mass spectrometry to have a molecular formula $C_{30}H_{50}O$ (calculated mol wt 426.3859); the molecular ion (M^+) was given at m/e 426.3819 (relative intensity 20%), with other principal ions at m/e 411.3595 (100%), 393.3492 (22%), 313.2574 (3%), 271.2403 (3%), 259.2088 (6%), 255.2117 (5%), 241.1910 (2%), and

TABLE I

Relative Retention Times and ΔR_{Ac}-Values of 5α-Euphane and 5α-Tirucallane Series Triterpene Alcohols Determined on 3% OV-17 Column

Compound	RRT[a] 3β-OH	RRT[a] 3β-OAc	ΔR_{Ac}[b]
5α-Euphane series			
Ia	0.89	1.07	1.20
Ib	0.74	0.89	1.20
IIa	1.17	1.40	1.20
IIb	0.96	1.16	1.21
5α-Tirucallane series			
Ic	1.01	1.22	1.21
Id	0.84	1.01	1.20
IIc	1.32	1.59	1.20
IId	1.08	1.31	1.21

[a]Retention time for sitosterol (30 min) is taken as 1.00.

[b]ΔR_{Ac}-value is expressed by the ratio of RRT of the acetate to RRT of the free alcohol.

229.1944 (4%). The triterpene alcohol showed on analytical TLC a mobility identical with that of eupha-7,24-dienol(IIa).

Hydrogenation of the Unknown Triterpene Acetate

Hydrogenation of the triterpene acetate afforded the dihydro acetate (RRT 1.31), mp 131-133 C (fine needles). IR of the dihydro acetate still showed the absorptions at 840 and 821 cm^{-1} related to a trisubstituted double bond; however, the intensity of these bands was found weaker than that observed for the acetate mentioned above. Hydrolysis of the dihydro acetate gave free alcohol (RRT 1.08), mp 95.5-97 C (plates). MS = m/e 428 (M^+, 19%), 413 (100%), 395 (77%), 315 (3%), 299 (3%), 297 (3%), 273 (9%), 259 (7%), 255 (4%), and 241 (5%).

Isomerization of the Dihydro Derivative of the Unknown Triterpene Acetate by HCl

A solution of the dihydro acetate (43 mg; RRT 1.31) in $CHCl_3$ (20 ml) was treated with a stream of dry HCl at 0 C for 3 hr in a similar manner as described by Dawson et al. (5). GLC of the isomerized product (40 mg) gave a major component peak at RRT 1.01 (relative abundance 76%), with three other minor component peaks at RRT 0.83 (17%), 1.07 (5%), and 1.31 (2%). Crystallization of this isomerized product afforded flat needles (22 mg) with mp 146-148 C (GLC: RRT 1.01, 97%; RRT 1.07, 3%). IR of the acetate showed no absorptions related to trisubstituted double bonds. MS = m/e 470 (M^+, 15%), 455 (75%), 410 (2%), 395 (100%), 357 (1%), 315 (2%), 313 (2%), 301 (5%), 299 (5%), 297 (2%), 255 (6%), and 241 (13%).

Tirucalla-8,24-dienol (Fig. 1, Ic) and Its Dihydro Derivative (Id)

An authentic specimen of tirucalla-8,24-dienol showed M^+ at m/e 426 (34%), with other ions at m/e 411 (100%), 393 (52%), 313 (3%), 297 (4%), 273 (7%), 271 (6%), 259 (17%), 255 (8%), and 241 (13%) in the mass spectrum. Hydrogenation of its acetate (mp 161-163 C, fine needles) (lit. mp 163.5 C [6]) gave tirucall-8-enyl (Id) acetate, mp 146-148 C (fine needles) (lit. mp 147-149 C [6]). IR, MS, and PMR spectra, as well as RRT in GLC and melting point of this acetate, were identical with those observed for the isomerized dihydro acetate derived from the unknown alcohol described above.

Eupha-7,24-dienol (IIa) and Its Dihydro Derivative (IIb)

Eupha-7,24-dienyl (IIa) acetate isolated from tea seed oil (1) showed mp 146-147 C (fine needles), $[\alpha]_D^{21}$ +10° (c 0.81) (lit. mp 146.5-147.5 C, $[\alpha]_D$ +11° [7]). IR showed the presence of trisubstituted double bonds (841, 832, and 816 cm^{-1}). Hydrolysis of the acetate, gave free alcohol, mp 107-110 C (fine needles) (lit. mp 111-113 C [7]). MS = m/e 426 (M^+, 22%), 411 (100%), 393 (48%), 313 (13%), 297 (4%), 273 (5%), 271 (9%), 259 (12%), 255 (8%), and 241 (6%). Hydrogenation of the IIa acetate gave euph-7-enyl (IIb) acetate, mp 138-140 C (fine needles) (lit. mp 137-139 C [7], 134-135 C [8]), which on hydrolysis gave free alcohol IIb. The IR absorptions attributable to the trisubstituted double bond (838, 821, and 812 cm^{-1}) were found weaker than those observed for the IIa acetate described above. MS = m/e 428 (M^+, 8%), 413 (100%), 395 (70%), 315 (2%), 299 (7%), 273 (20%), 259 (14%), 255 (6%), and 241 (10%).

Isomerization of Euph-7-enyl (IIb) Acetate by HCl

Euph-7-enyl (IIb) acetate (100 mg, >99% pure by GLC, RRT 1.16) in $CHCl_3$ (20 ml) was treated with dry HCl as described above. The isomerized product (98 mg) was shown in direct GLC to consist of a component with RRT 0.89 (relative abundance 86%) and two minor components with RRT 0.78 (10%) and 0.98 (4%). No detectable amount of the starting material (IIb-acetate, RRT 1.16) was found in the GLC. The product on recrystallization gave fine needles (70 mg) with mp 126-127 C (GLC: RRT 0.89, 96%; RRT 0.98, 4%). The product is regarded as a Δ^8-isomer of euph-7-enyl (IIb) acetate, i.e., euph-8-enyl (Ib) acetate

(lit. mp 124-127 C [5], 124-126 C [9]). IR of the Ib acetate showed no absorptions correlated to trisubstituted double bonds. MS = m/e 470 (M^+, 22%), 455 (86%), 410 (2%), 395 (100%), 357 (2%), 315 (2%), 313 (1%), 301 (4%), 299 (4%), 297 (4%), 255 (5%), and 241 (11%).

RESULTS AND DISCUSSION

The mobility in TLC and ΔR_{Ac}-value (Table I) for the unknown alcohol isolated from tea seed oil are indicative of the presence of the usual 30 (4α), 31 (4β)-dimethyl-5α-stan-3β-ol grouping in the ring A (10,11). The high resolution mass spectrum indicates a molecular formula $C_{30}H_{50}O$ for the alcohol. The presence of the ions at m/e 313.2574 ($M^+ - C_8H_{15}$ [side chain] - 2H, requires 313.2530) and m/e 259.2088 ($M^+ - C_8H_{15} - C_3H_6$ [part of ring D] - CH_2, requires 259.2061) shows that this triterpene alcohol possesses a monounsaturated side chain (12) and also a monounsaturated skeleton with the additional C-32 methyl group (13). Both the double bonds were found as trisubstituted since the IR absorptions related to trisubstituted double bonds and observed for the unknown alcohol at 840 and 821 cm^{-1} were still observed as weakened bands for its dihydro derivative. The PMR spectrum of the unknown alcohol showed two olefinic protons (δ 5.12 and 5.16), of which the one (δ 5.12) must be on the side chain isopropylidene group since it disappeared in the spectrum of the dihydro alcohol; signals due to two vinylic methyls (26,27-methyls) (14) were also observed at δ 1.62 and 1.70 in the spectrum of the unknown alcohol. The presence of an axial proton at C-3 with a broad multiplet (δ 3.22) is also in support of the 3β-configuration of the hydroxy group (15). The spectrum of the alcohol also showed five tertiary methyl groups which must be located in the ring system with the singlets at δ 0.79 (3H), 0.82 (3H), 0.87 (3H), and 0.99 (6H). The chemical shifts of these methyl signals are found nearly identical with those observed for 5α-eupha-7,24-dien-3β-ol (Fig. 1, IIa). These facts may be rightly interpreted by regarding the ring system of the unknown alcohol as identical with that of IIa, the ring system of euphane-tirucallane series with Δ^7-bond (II).

The five tertiary methyl groups of the unknown alcohol showed the signals at δ(LSR) 11.00 (3H), 10.14 (3H), 4.49 (3H), 2.09 (3H), and 1.58 (3H) after the normalization of the LIS in the spectrum recorded in the presence of a molar equivalent of $Eu(DPM)_3$. Careful preliminary experiments in which the spectra were measured with every amount of $Eu(DPM)_3$ added have revealed that these methyl signals are associated with the signals at δ 0.87, 0.99, 0.79, 0.99, and 0.82, respectively, in the spectrum measured in the absence of the LSR. The signals at δ(LSR) 11.00 and 10.14 are correlated with 31 (4β)-methyl, the methyl group nearest to the 3β-OH, and 30 (4α)-methyl groups, respectively (3,4,16). The third methyl signal from the lower field at δ(LSR) 4.49 is attributable to the 19-methyl group. The Δδ-value ($\Delta\delta = \delta[LSR] - \delta$) of 19-methyl signal (Δδ 3.70) gives strong support to admit that the position of the trisubstituted double bond of the unknown alcohol is Δ^7 in the ring system because 5α-lanost-7-en-3β-ol (Fig. 1, Vb; Δδ 3.70), 5α-eupha-7,24-dien-3β-ol (IIa,Δδ 3.75), and 5α-euph-7-en-3β-ol(IIb,Δδ 3.76) also show similar Δδ-values for the corresponding methyl signal and there is no difference in the rings A and B chemistry between the triterpenes of lanostane and euphane-tirucallane series. Furthermore, the triterpenes with $\Delta^{9(11)}$- or Δ^8-bond indicate somewhat larger Δδ-values for the methyl signal: 24-methyl-5α-lanost-9(11)-en-3β-ol (IIIe, Δδ 3.87), 5α-lanosta-8,24-dien-3β-ol (Id, Δδ 3.91), 5α-euph-8-en-3β-ol (Ib,Δδ 3.95), and 5α-tirucall-8-en-3β-ol (Id,Δδ 3.90). The possibility of $\Delta^{9(11)}$-bond for the skeletal double bond of the unknown alcohol is, therefore, excluded.

The remaining two methyl signals with δ(LSR) 1.58 (Δδ 0.76) and 2.09 (Δδ 1.10) are attributable to 18- and 32-methyl groups, respectively, because it is seen from the Δδ-values listed in Table II that, among 18- and 32-methyl groups, the β-oriented one shows a larger Δδ-value than the other α-oriented one; the Δδ-values for the triterpenes are 18-methyl (β-oriented) > 32-methyl (α-oriented) on the lanostane series and, on the other hand, 18-methyl (α-oriented) < 32-methyl (β-oriented) on the euphane-tirucallane series. The δ(LSR)-values of all five methyl signals in the ring system of the unknown alcohol are identical within the experimental error with those measured for IIa. This is fully explicable by the ring system of II (Fig. 1) for the unknown alcohol. When the C-9 proton of the unknown alcohol is β-oriented, it causes some difference in the PMR data described above between the unknown alcohol and the reference eupha-7,24-dienol (IIa), of which the α-orientation of C-9 proton has already been ascertained (5,8).

The unknown alcohol, thus shown to possess the ring system II (Fig. 1), and its dihydro derivative indicate that the MS, IR, and PMR (without the LSR) spectral patterns are nearly identical with those of eupha-7,24-dienol (IIa) and its dihydro derivative (IIb), respecitvely,

TABLE II

Chemical Shifts (δ, ppm)[a] and Normalized Lanthanide-induced Shifts (δ[LSR], ppm)[b] of Proton Signals of 5α-Euphane, 5α-Tirucallane, and 5α-Lanostane Series of Triterpene Alcohols

Compound	C-3 substituent	Methyl groups									
		18	19	30	31	32	21	26,27	3β-OAc	3α-CH	Others
5α-Euphane series											
Eupha-8,24-dienol(Ia)	3β-OAc	0.76	0.98	0.88	0.88	0.88	0.85[e]	1.62,1.69	2.05	4.50*m*	5.08*t*(24-CH)
	3β-OH	0.76	0.97	1.00	0.81	0.88	f	1.62,1.69		3.20*m*	5.08*t*(24-CH)
Euph-8-enol (Ib)	3β-OAc	0.76	0.99	0.88	0.88	0.88	0.85[e]	0.87*d*	2.05	4.56*m*	
	3βOH[b]	1.48	4.92	10.32	11.00	1.96	1.09*d*	0.89*d*			
	3βOH	0.77	0.97	1.02	0.83	0.89	f	0.88*d*			
	$\Delta\delta$[c]	0.71	3.95	9.30	10.13	1.07		0.01			
Eupha-7,24-dienol(IIa)	3β-OAc[d]	0.82	0.78	0.96	0.85	1.00	f	1.62,1.70	2.05	4.56*m*	5.10*m*(24-CH),5.21*m*(7-CH)
	3β-OH[b]	1.58	4.51	10.18	11.00	2.10	1.16*d*	1.62,1.68			5.21*m*(24-CH),6.89*m*(7-CH)
	3β-OH	0.81	0.76	0.98	0.87	0.98	0.89[e]	1.62,1.70		3.25*m*	5.10*m*(24-CH),5.23*m*(7-CH)
	$\Delta\delta$	0.77	3.75	9.20	10.13	1.12		0 -0.02			0.11(24-CH) 1.66(7-CH)
Euph-7-enol(IIb)	3β-OH[b]	1.61	4.52	10.19	11.00	2.13	1.16*d*	0.91*d*			6.91*m*(7-CH)
	3β-OH	0.83	0.76	0.99	0.88	0.99	0.89[e]	0.88*d*		3.22*m*	5.24*m*(7-CH)
	$\Delta\delta$	0.78	3.76	9.20	10.12	1.14		0.03			1.67(7-CH)
5α-Tirucallane series											
Tirucalla-8,24-dienol(Ic)	3β-OAc[d]	0.76	0.99	0.89	0.89	0.89	0.96[e]	1.62,1.69	2.05	4.48*m*	5.11*m*(24-CH)
Tirucall-8-enol(Id)	3β-OAc	0.77	0.99	0.89	0.89	0.89	0.96[e]	0.87*d*	2.06	4.53*m*	
	3β-OH[b]	1.50	4.87	10.37	11.00	1.94	1.16*d*	0.95*d*			
	3β-OH	0.77	0.97	1.02	0.82	0.88	f	0.87*d*			
	$\Delta\delta$	0.73	3.90	9.35	10.12	1.06		0.08			
Tirucalla-7,24-dienol(IIc)	3β-OAc[d]	0.82	0.79	0.94	0.87	0.99	0.93[e]	1.62,1.70	2.05	4.54*m*	5.10*m*(24-CH),5.24*m*(7-CH)
	3β-OH[b]	1.58	4.49	10.14	11.00	2.09	1.19*d*	1.74,1.80			5.31*m*(24-CH),6.90*m*(7-CH)
	3β-OH	0.82	0.79	0.99	0.87	0.99	0.94[e]	1.62,1.70		3.22*m*	5.12*m*(24-CH),5.26*m*(7-CH)
	$\Delta\delta$	0.76	3.70	9.15	10.13	1.10		0.12,0.10			0.19(24-CH) 1.64(7-CH)
Tirucall-7-enol(IId)	3β-OH	0.82	0.76	0.98	0.87	0.98	f	0.87*d*		3.22*m*	5.24*m*(7-CH)
5α-Lanostane series											
24-Methyllanost-9(11)-enol (IIIe)	3β-OH[b]	1.70	4.92	10.26	11.00	1.72	1.30*d*	1.04*d*[g]			0.99*d*(28-CH_3)[g],6.96*m*(11-CH)
	3β-OH	0.66	1.05	1.00	0.82	0.75	f	0.87*d*		3.25*m*	5.24*m*(11-CH)
	$\Delta\delta$	1.04	3.87	9.26	10.18	0.97		0.17			1.72(11-CH)
Lanosta-8,24-dienol(IVa)	3β-OH[b]	1.79	4.92	10.24	11.00	1.79	1.31*d*	1.75,1.84			5.38*m*(24-CH)
	3β-OH	0.70	1.01	1.01	0.82	0.88	f	1.62,1.69		3.26*m*	5.11*m*(24-CH)
	$\Delta\delta$	1.09	3.91	9.23	10.18	0.91		0.13,0.15			0.27
Lanost-7-enol(Vb)	3β-OH[b]	1.74	4.59	10.04	11.00	1.85	1.32*d*	1.06*d*			6.92*m*(7-CH)
	3β-OH	0.65	0.89	1.00	0.90	1.00	f	0.88*d*		3.26*m*	5.21*m*(7-CH)
	$\Delta\delta$	1.09	3.70	9.04	10.10	0.85		0.18			1.71(7-CH)

though between these two isomers apparent differences are recognized in GLC, melting point, and $[\alpha]_D$. These facts may be explained by giving the side chain c (Fig. 1), C-20 epimer (20αH. 20*S*) of a (20βH, 20*R*), to the unknown alcohol since between 5α-eupha-8,24-dien-3β-ol (Ia) and 5α-tirucalla-8,24-dien-3β-ol (Ic), a known couple of C-20 epimers, similar correlations in the physical characteristics can be observed. That the unknown alcohol has the side chain c is justified also by the PMR spectroscopy with the LSR. Three triterpenes of euphane series, Ib, IIa, and IIb (Fig. 1), afforded Δδ -0.02 - 0.03 associated with 26,27-dimethyl signal(s), whereas the Δδ 0.08 was observed for the dimethyl signal of tirucall-8-enol (Id). The difference in Δδ-values between these two series is thought to have arisen from the inverted configuration at C-20. Then, the unknown alcohol exhibiting the Δδ 0.10 and 0.12 for the dimethyl signals is considered to carry the side chain of tirucallane series, i.e., c. Accordingly, the unknown alcohol isolated from tea seed oil may be concluded to have the structure of IIc (Fig. 1), 5α-tirucalla-7,24-dien-3β-ol, a new triterpene alcohol from natural sources.

The structure IIc given for the new alcohol is in full accord with the result of HCl isomerization. As euph-7-enol gives its Δ^8-isomer by treatment with HCl in $CHCl_3$ (5), so isomerization of euph-7-enyl (IIb) acetate in this study also indicates the formation of the acetate of the Δ^8-isomer (Ib). On the other hand, the dihydro acetate of the new alcohol on HCl treatment afforded a compound identical with the authentic specimen of tirucall-8-enyl (Id) acetate, and therefore the new alcohol must be a Δ^7-isomer (IIc) of 5α-tirucalla-8,24-dien-3β-ol (Ic).

It should be noted here that acid-catalyzed isomerization is reversible for lanost-8-enol (IVb) (2,17,18) and 32 (14α)-methylcholest-7-enol (18). These compounds yield an equilibrium mixture of Δ^7- and Δ^8-isomers by HCl treatment. On the other hand, as has already been shown (5) and also as described in the experimental section, the Δ^7-compounds of euphane and tirucallane series by HCl treatment under a very mild condition yield principally the Δ^8-isomers with several minor products. Attempted HCl treatment of euph-8-enol (Ib) under mild conditions gave no evidence for the formation of the Δ^7-isomer by GLC. Under the more violent conditions of acid isomerization, euph-8-enyl acetate is known to yield isoeuph-13(17)-enyl acetate rather than the Δ^7-isomer (19).

Gas chromatographic correlations of the tri-

[a]60 MHz, 80 μmol of triterpene in 0.4 ml of $CDCl_3$, 28 C, internal tetramethylsilane = 0 ppm; each signal was a singlet unless otherwise stated, in which case the multiplicity is given after the chemical shift: *d* = doublet (*J* 6.0 Hz), *t* = triplet (*J* 6.0-7.2 Hz), and *m* = multiplet.

[b]Normalized lanthanide-induced shift: 60 MHz, 80 μmol of triterpene alcohol in 0.4 ml of $CDCl_3$, molar ratio of $Eu(DPM)_3$ to alcohol is 1, 28 C, internal tetramethylsilane = 0 ppm; *d* = 21-CH_3, *J* 3.6-4.8 Hz; 26,27-methyls, *J* 5.4-6.6 Hz.

[c]Δδ-Value = δ [LSR] - δ, correlated to the 3β-OH data.

[d]Measured on a 100 MHz instrument under the condition described in footnote "a".

[e]Shoulder peak, part of a doublet signal.

[f]The presence of the shoulder peak is not clear.

[g]These doublets could not be sharply separated from one another, presumably due to the presence of C-24 epimers.

TABLE III

C-20 Epimeric (5α-Tirucallane/5α-Euphane) and Skeletal Isomeric (Δ^7/Δ^8) Separation Factors of Triterpene Alcohols on 3% OV-17 Column

C-20 Epimeric (5α-Tirucallane/5α-Euphane) Separation Factor

Compound compared		Separation factor 3β-OH	Separation factor 3β-OAc
Δ^8	Ic/Ia	1.13	1.14
	Id/Ib	1.14	1.13
Δ^7	IIc/IIa	1.13	1.14
	IId/IIb	1.13	1.13

Skeletal Isomeric (Δ^7/Δ^8) Separation Factor

Compound compared	Separation factor 3β-OH	Separation factor 3β-OAc
5α-Euphane series		
IIa/Ia	1.31	1.31
IIb/Ib	1.30	1.30
5α-Tirucallane series		
IIc/Ic	1.31	1.30
IId/Id	1.29	1.30

terpenes of euphane and tirucallane series furnish strong support for structure IIc of the new alcohol. Table I shows RRT determined on 3% OV-17 column and ΔR_{Ac}-values (10,11), the ratio of the RRT of the acetate to the RRT of the corresponding free alcohol, of the two series of triterpenes. The ΔR_{Ac}-values of these triterpenes are essentially similar to those observed on 5α-lanostane series compounds (10,11), as expected inasmuch as there is no difference in the spacial configurations of rings A and B between these series. Table III shows C-20 epimeric (5α-tirucallane/5α-euphane) and skeletal isomeric (Δ^7/Δ^8) separation factors of the triterpenes on 3% OV-17 column calculated from the retention data listed in Table I. Triterpenes of tirucallane series are more strongly retained than those of euphane series on OV-17 stationary phase. Ikan and Gottlieb (20) also observed a similar tendency between the two series of triterpenes as their trimethylsilyl ether derivatives on XE-60 column.

Many meliane (the group of compounds having a tirucallane type skeleton with an oxygenated side chain) and meliacan (the group of compounds possessing an apo-tirucallane type skeleton) series of oxygenated triterpenes have recently been ascertained to be present in the Meliaceae and the related Rutaceae and Simaroubaceae species (21-27). The new alcohol (IIc) isolated from tea seed oil in this study is just a compound which has been considered as a possible biogenetic precursor of these oxygenated tetracyclic triterpenes (21,23,26); nevertheless, its occurrence in natural sources has not yet been known, though the previous existence of the tirucalla-7,24-diene structure in the form bearing a carboxylic group at C-20 is known as one of the elemolic acids (3α-hydroxy-5α-tirucalla-7,24-dien-21-oic acid, Fig. 1, VIf) (22). The presence of the new triterpene alcohol (IIc) is ascertained also in the seed oils of two other theaceous plants, *Camellia japonica* and *Camellia Sasanqua* (unpublished data).

ACKNOWLEDGMENTS

Izome Oil Chemical Industries Co. supplied the tea seed oil, G. Ourisson provided euphol, and R. Ikan supplied tirucallol. T. Wainai and C. Hirai provided the facilities of the measurements of PMR spectra, T. Miyao helped us in optical rotation measurements, and Y. Hirano and K. Uchikawa gave technical assistance. Y. Toyama contributed comments and advice.

REFERENCES

1. Itoh, T., T. Tamura, and T. Matsumoto, Lipids 9:173 (1974).
2. Itoh, T., T. Tamura, and T. Matsumoto, Ibid. 10:454 (1975).
3. Buckley, D.G., G.H. Green, E. Ritchie, and W.C. Taylor, Chem. Ind. 1971:298.
4. Itoh, T., T. Tamura, and T. Matsumoto, Steroids 27:275 (1976).
5. Dawson, M.C., T.G. Halsall, E.R.H. Jones, and P.A. Robins, J. Chem. Soc. 1953:586.
6. Hains, D.W., and F.L. Warren, Ibid. 1949:2554.
7. Heilbron, I.M., E.R.H. Jones, and P.A. Robins, Ibid. 1949:444.
8. Lawrie, W., W. Hamilton, F.S. Spring, and H.S. Watson, Ibid. 1956:3272.
9. Dawson, M.C., T.G. Halsall, E.R.H. Jones, G.D. Meakins, and P.C. Phillips, Ibid. 1956:3172.
10. Itoh, T., T. Tamura, T. Iida, and T. Matsumoto, Steroids 23:687 (1974).
11. Itoh, T., T. Tamura, T. Iida, and T. Matsumoto, Ibid. 26:93 (1975).
12. Wyllie, S.G., and C. Djerassi, J. Org. Chem. 33:305 (1968).
13. Goad, L.J., B.L. Williams, and T.W. Goodwin, Eur. J. Biochem. 3:232 (1967).
14. Scallen, T.J., and W. Krueger, J. Lipid Res. 9:120 (1968).
15. Bhacca, N.S., and D.H. Williams, "Application of NMR Spectroscopy in Organic Chemistry–Illustrations from the Steroid Field," Holden-Day, Inc., San Francisco, CA, 1964, p. 78.
16. Danieli, B., G.P. Forni, G. Palmisano, G. Rainoldi, and G. Saverini, Chem. Ind. 1974:748.
17. Marker, R.E., E.L. Wittle, and L.M. Mixon, J. Am. Chem. Soc. 59:1368 (1937).
18. Gaylor, J.L., C.V. Celwiche, and A.C. Swindell, Steroids 8:353 (1966).
19. Fieser, L.F., and M. Fieser, "Steroids," Reinhold Publishing Co., New York, NY, 1959, p. 389.
20. Ikan, R., and R. Gottlieb, Isr. J. Chem. 8:685 (1970).
21. Bevan, C.W.L., D.E.U. Ekong, T.G. Halsall, and P. Toft, J. Chem. Soc. C 1967:820.

22. Cotterrell, G.P., T.G. Halsall, and M.J. Wrigleworth, Ibid. 1970:739.
23. Cotterrell, G.P., T.G. Halsall, and M.J. Wrigleworth, Ibid. 1970:1503.
24. Buchanan, J.G.St.C., and T.G. Halsall, Ibid. 1970:2280.
25. Basak, S.P., A. Islam, and D.P. Chakraborty, J. Indian Chem. Soc. 47:501 (1970).
26. Lavie, D., and E.C. Levy, Tetrahedron 27:3941 (1971).
27. Merrien, A., and J. Polonsky, Chem. Commun. 1971:261.

[Received July 30, 1975]

A Guide for Authors is Located in Lipids 11(January):85(1976)