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EPOXIDE CONFIGURATION IN 3β-ACETOXY-7α-HYDROXY-14,15-EPOXY-APOTIRUCALLANE PREPARED FROM TIRUCALLA-7,24-DIEN-3-ONE. X-RAY ANALYSIS

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Abstract—Epoxidation of 3β - Acetoxy - 7α - hydroxy - Δ^{14} - apotirucallane, prepared from Δ^7-tirucallone, afforded the corresponding 14α,15α epoxide. The α-configuration of the epoxide function has been established by single-crystal X-ray analysis, representing the first such study of a tirucallol derivative with an intact side-chain. Epoxidation of Apotirucalla - 3,7 - dione gave the corresponding 14α,15α - epoxide as well. Eight new tirucallol derivatives were described.

Previous investigations on the cytotoxic limonoids from the Himalayan Meliaceae species *Aphanamixis graddifolia* Bl. have shown that an intact steroid D-ring system bearing a 14β,15β - epoxide was an important structural requirement for inhibiting growth of the murine P388 lymphocytic leukemia cell line.[1] Thus, aphanastatin **1**, amoorastatin **2**, 12-hydroxy amoorastatin **3** and sendanin **4** display substantial P388 cell growth inhibition. In contrast, amoorastatone **5**, in which the 14,15-epoxide has been replaced by the product of rearrangement, namely a 15-oxo system, was considered marginally inactive.[1] Apparently the 14,15β-epoxide is a very definite requirement for inhibition of neoplastic (P388) cell growth and is reminiscent of an analogous structural modification and loss of activity observed with compounds of the bufadienolide type.[2] These findings suggested that the introduction of a 14,15-epoxide function in an inactive tetracyclic triterpene might afford a cytotoxic compound. The title compound was therefore prepared from Δ^7-tirucallone **6**, the proposed biogenetic precursor of the limonoids and quassinoids.[3,4] The α-configuration of the epoxide function was established unambiguously by single-crystal X-ray analysis.

Preparation of epoxide **11**. Tirucalla - 7,24 - dien - 3 - one **6**, isolated from the Guyanean Simaroubaceae species *Simarouba amara*,[5] was converted into the known 24,25 - dihydro - Δ^7 - tirucallol **7**,[5,6] whose acetate **8** afforded upon epoxidation the 7α,8α-epoxide **9**. The latter underwent "apo-rearrangement"[7,8] upon exposure to boron trifluoride etherate to give the apo-compound **10** which with monoperphthalic acid in ether (in the presence of powdered Na_2HPO_4) afforded in good yield the 14,15-epoxide **11**. Its mass spectrum showed the molecular ion peak at m/z 502 and displayed fragmentation ions at m/z 487 ($M^{+\cdot} - 15$), 469 ($M^{+\cdot} - 15 - 18$) and a significant ion at m/z 209, which probably arises by a McLafferty type rearrangement followed by cleavage of the C-12, C-13 bond.

Epoxide **11** proved to be extremely sensitive to acids and afforded the rearranged 15-keto compound even when exposed briefly to the weakly acidic phthalic acid and epoxidation was carried out in the presence of a buffer; the filtrate from the reaction mixture was washed immediately with saturated $NaHCO_3$ solution. The rearranged 15-keto compound, although displaying similar chromatographic and mass spectrometric behaviour (base peak at m/z 209.1880, $C_{14}H_{25}O$) to that of epoxide **11**, could be easily differentiated from the latter by its circular dichroism spectrum [$\Delta\epsilon$ − 3.73 (295 nm) and $\Delta\epsilon$ − 3.71 (303 nm) in dioxane].

Preparation of epoxides **14** *and* **16**. Treatment of 24,25 - dihydro - Δ^7 - tirucallone **12** (obtained by catalytic hydrogenation of **6**) with perphthalic acid gave a mixture of products with upon chromatography on alumina afforded directly the rearranged apo-compound **13**. Epoxidation of the latter with monoperphthalic acid gave in good yield the 14,15-epoxide **14**. Compound **13**, when exposed to Jones' reagent, gave the diketo - apo - compound **15**, which afforded upon the usual epoxidation conditions 3,7 - diketo - 14,15 - epoxy - apotirucallane **16**, identical with the product obtained from Jones' oxidation of **14**.

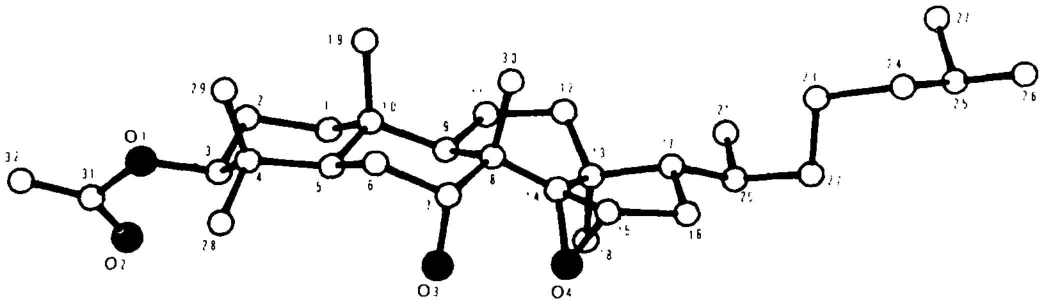
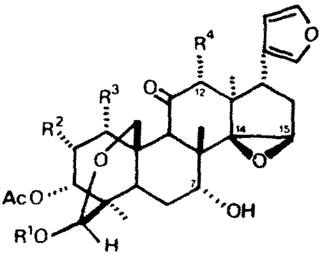
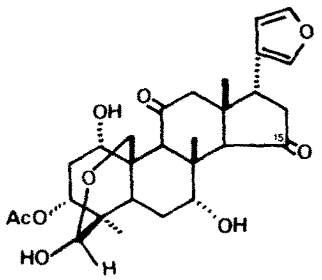


Fig. 1. A perspective view and atom numbering of the title molecule.



1 : $R^1 = COCH(CH_3)C_2H_5$; $R^3 = Ac$
$R^2 = R^4 = OH$
2 : $R^1 = R^2 = R^4 = H$; $R^3 = OH$
3 : $R^1 = R^2 = H$; $R^3 = R^4 = OH$
4 : $R^1 = Ac$; $R^2 = H$, $R^3 = OH$; $R^4 = OAc$



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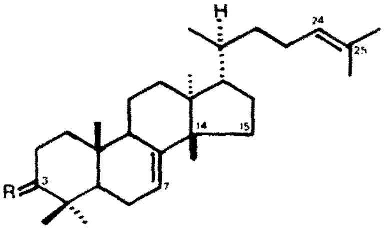
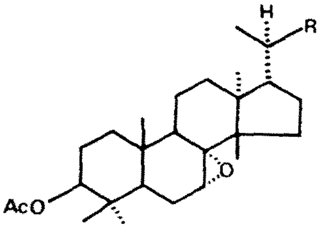
Epoxide **11** was subjected to X-ray diffraction studies which showed the epoxide configuration to be α. A perspective view of the molecule **11** with atom numbering is illustrated in Fig. 1. The ring junctions are: A/B, *trans*; B/C, *trans*; C/D, *cis*. The side chain is in an extended conformation as in 5α-cholest - 2 - ene.[9] The positions and configurations of the substituents are 3β-OAc, 4β-CH_3, 4α-CH_3, 7α-OH, 8β-CH_3, 10β-CH_3, 14α, 15α-epoxy and 17 α-side chain (C_8H_{17}) possessing the S configuration of C-20. The 7α-hydroxy group is hydrogen bonded to the epoxy group; O(3)... O(4) distance: 2.622(16) Å, H(03)...O(4) distance: 1.952(11) Å and the O(3)–H(O3).. O(4) angle is 125.7 (7°).

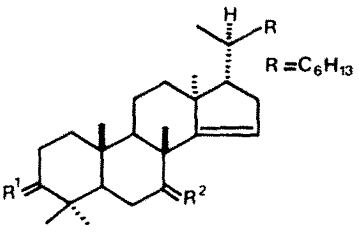
The epoxide configuration in compound **14** is assumed



6 R = O
7 R = OH, H
8 R = OAc, H
12 R = O
(7, 8, 12) 24,25-dihydro

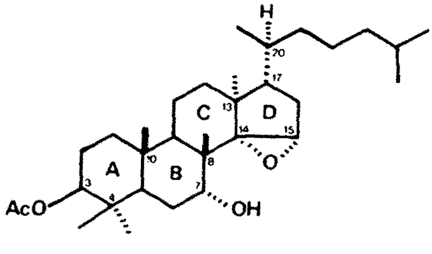


9 $R = C_6H_{13}$

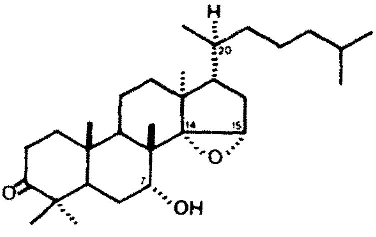


$R = C_6H_{13}$

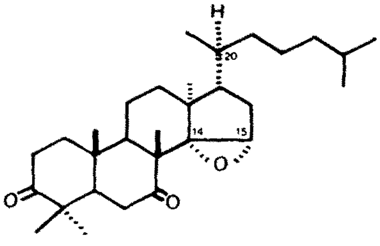
10 R^1 = OAc, H ; R^2 = OH, H
13 R^1 = O ; R^2 = OH, H
15 $R^1 = R^2$ = O



11



14 $R = C_6H_{13}$



16

to be α based on our experience with epoxidation of **10** which afforded the 14α,15α-epoxide **11**. The α-configuration of the epoxide in these two compounds was expected on the basis of mechanistic considerations (7α-OH direction of epoxidation). Interestingly, compound **15** when treated with perphthalic acid afforded the epoxide **16** possessing the α-configuration as well (this was established by its identity with the Jones' oxidation product of **14**). This result shows that steric factors disfavouring β-epoxidation are as important as hydrogen bonding in determining the stereochemical outcome of the epoxidation.

The title compound does not inhibit growth of the P388 leukemia cell line nor cell transformation induced by Rous sarcoma visus.[10] This negative result suggests that our initial assumption that a 14,15-epoxide function might confer cytotoxicity to an otherwise inactive triterpene was untenable. It appears that epoxide configuration as well as other structural features are of importance for biological activity in this type of compounds.

To our knowledge this study represents the first X-ray analysis of a tirucallol derivative with an intact side chain. Compounds **8**–**16** have not been previously described.

EXPERIMENTAL

M. ps were determined on a Kofler melting point apparatus and uncorrected. Optical rotations were determined at 22° on a Roussel-Jouan Quick polarimeter for chloroform solutions. Circular dichroism measurements were made with a Roussel-Jouan Dichrographe II. Electron impact mass spectra were taken on a MS 50-AEI spectrometer. The ^1H NMR spectra were recorded with a Varian T60 and with a Bruker HXE 90 spectrometers in deuteriochloroform, using TMS as an internal standard.

3β-Acetoxy-Δ⁷-tirucallane **8**

24,25 - Dihydro - Δ^7 - tirucallol **7**[5] (640 mg) in acetic anhydride (3 ml) and pyridine (3 ml) was set aside at room temp overnight. Working up in the usual manner gave a product (670 mg) which was homogenous on tlc. Crystallisation from methanol gave the acetate **8** as colourless prisms, m.p. 123–125° (Found: C, 81.35; H, 11.44. $C_{32}H_{54}O_2$ requires: C, 81.6; H, 11.56%). ^1H NMR: m, δ 4.53 and 5.30 ppm (H-3 and H-7, respectively) and s, δ 2.03 ppm (OAc).

3β-Acetoxy-7α,8α-epoxy-tirucallane **9**

The acetate **8** (171 mg) was dissolved in dry ether (3 ml), the solution was cooled to −3°, and an etheral solution of monoperphthalic (0.5 N; 6 ml) was added. The solution was kept for 30 hr at −2° and then poured into an aqueous solution of sodium disulfite. Extraction with ether yielded a product (170 mg) which was chromatographed on silica gel (60 Merck) (17 g). Elution with 5% ether in hexane afforded starting material (12 mg) and then the 3β - acetoxy - 7α,8α - epoxide **9** (92 mg) as prisms (from methanol). M.p. 158–160°, $[\alpha]_D^{22}$ −60.4° (c 1.23). $C_{32}H_{54}O_3$: $M^{+\cdot}$ at *m/z* 486. ^1H NMR: m, δ 4.36 and 2.80 ppm (H-3 and H-7, respectively).

3β-Acetoxy-7α-hydroxy-Δ¹⁴-apotirucallane **10**

The epoxide **9** (80 mg) was dissolved in dry benzene (2 ml) and boron trifluoride–ether complex (3 drops) was added. The solution was stirred for 15 min and poured into hydrogen carbonate solution. Extraction of the reaction mixture with ether afforded a crystalline solid which was purified by column chromatography on silica gel (8 g). Elution with 10% ether in hexane gave the pure apo-compound **10** which was crystallised from methanol. M.p. 136–138°, $[\alpha]_D^{22}$ −72.2° (c 1.24). $C_{32}H_{54}O_3$ $M^{+\cdot}$ at *m/z* 486. ^1H NMR: m, δ 3.89, 4.52 and 5.36 ppm (H-7, H-3 and H-15, respectively).

3β-Acetoxy-7α-hydroxy-14α,15α-epoxy-apotirucallane **11**

Powdered Na_2HPO_4 (60 mg) was added to a solution of the apo-compound **10** (60 mg) in dry ether (2 ml). The mixture was cooled to 0° and an ethereal solution of monoperphthalic acid (0.5 N, 3 ml) was added. After setting aside at 0° for 24 hr, the reaction mixture was diluted with ether. The solids were filtered off and the filtrate was rapidly washed with an aqueous solution of sodium hydrogen carbonate (3 times) and then with water. Evaporation of the solvent yielded a crystalline solid (70 mg) which was recrystallised from ether–hexane to give the epoxide **11**, m.p. 188–190°, $[\alpha]_D^{22}$ −20.3° (c 0.94). (Found: C, 76.00; H, 10.77. $C_{32}H_{54}O_4$ requires: C, 76.44; H, 10.83%).

X-Ray crystallographic analysis of epoxide **11**

A small white crystal, grown from ether (size 0.3 × 0.1 × 0.1 mm) was mounted on a Philips PW 1100 diffractometer with graphite monochromated MoKα radiation. Unit-cell dimensions and orientation matrix were obtained by least-squares from the setting angles of 25 reflexions. The intensities of reflexions with $\theta < 23°$ were measured by an $\omega/2\theta$ scan at a scan rate of 0.0125° s^{-1} and a scan angle of 1.0°. Background measurements of 15 s were made on both sides of each scan. Lorentz and polarisation corrections were applied to 1070 reflexions with $I < 3\sigma(I)$.

The structure was solved by the use of the Multan program.[11] Calculated from 317 E's > 1.45 and with the reflexions 3.10.0, 28.5.0 and 0.1.3 taken as origin, the E map with the best figure of merit revealed 14 of the nonhydrogen atoms. With the E recycling procedure[12] 12 other atoms were found and two successive Fourier syntheses led to the complete structure. In the refinement by full-matrix least-squares methods, the quantity $\Sigma \underline{w}(|\underline{F}_o| - |\underline{F}_c|)^2$ was minimized, where $\underline{w} = 1/\sigma^2(\underline{F})$. Only the O atoms and the C atoms of the skeleton were refined anisotropically. 26 H atoms, bonded directly to the skeleton, were included in their idealized positions with the equivalent isotropic thermal factors of the bonded carbons. All the other H atoms were revealed on difference Fourier synthesies and included in the structure factors calculations. The refinement converges to $\underline{R} = 0.089$ ($\underline{R}_w = 0.088$). Final atomic coordinates for the non-hydrogen atoms together with their estimated standard deviations (e.s.d.) are given in Table 1. Tables of calculated and observed structure amplitudes, anisotropic thermal parameters for the non-hydrogen atoms, and interatomic distances and angles are available on requuest from the authors.

7α-Hydroxy-Δ¹⁴-apotirucalla-3-one **13**

3 - Keto - Δ^7 - tirucallane **12** (600 mg) in dry ether (10 ml) was cooled to 0° and treated with ethereal perphthalic acid (0.9 N,

Table 1. Fractional atomic coordinates (× 10^4) with their estimated standard deviation in parentheses for heavy atoms

	X	Y	Z
O (1)	-1861 (4)	4894 (10)	2340 (22)
O (2)	-1775 (4)	5778 (13)	5339 (25)
O (3)	-431 (3)	2280 (8)	2792 (18)
O (4)	276 (3)	2527 (9)	2274 (20)
C (1)	-893 (5)	5530 (13)	811 (34)
C (2)	-1307 (5)	5639 (14)	942 (35)
C (3)	-1470 (5)	4718 (17)	2171 (35)
C (4)	-1417 (5)	3635 (16)	1162 (34)
C (5)	-991 (5)	3568 (12)	885 (25)
C (6)	-849 (5)	2546 (15)	43 (29)
C (7)	-440 (6)	2363 (12)	558 (33)
C (8)	-184 (5)	3239 (12)	-386 (32)
C (9)	-357 (5)	4286 (13)	253 (27)
C (10)	-777 (5)	4453 (12)	-230 (30)
C (11)	-109 (5)	5226 (12)	-511 (30)
C (12)	281 (5)	4952 (13)	-1065 (32)
C (13)	440 (5)	4128 (13)	356 (27)
C (14)	198 (5)	3126 (14)	330 (30)
C (15)	416 (6)	2199 (13)	268 (31)
C (16)	815 (5)	2490 (12)	88 (34)
C (17)	806 (5)	3669 (14)	-592 (30)
C (18)	506 (5)	4528 (13)	2577 (31)
C (19)	-867 (5)	4531 (13)	-2584 (31)
C (20)	1182 (5)	4219 (15)	-180 (32)
C (21)	1179 (5)	5398 (14)	-954 (30)
C (22)	1500 (6)	3680 (17)	-1077 (37)
C (23)	1488 (7)	3469 (18)	-3433 (39)
C (24)	1851 (7)	3138 (18)	-4510 (43)
C (25)	2178 (8)	3857 (22)	-4639 (47)
C (26)	2508 (8)	3250 (22)	-5341 (48)
C (27)	2090 (8)	4854 (23)	-5812 (48)
C (28)	-1553 (4)	2800 (13)	2757 (32)
C (29)	-1631 (5)	3518 (14)	-1048 (31)
C (30)	-182 (4)	3039 (13)	-2931 (29)
C (31)	-1958 (8)	5474 (23)	3984 (49)
C (32)	-2383 (6)	5756 (19)	3841 (41)

10 ml). Working up in the usual way gave a product (610 mg) which was a mixture of compounds. Column chromatography on alumina and elution with hexane containing increasing amounts of ether afforded starting material (60 mg) and the rearranged apo-compound **13** (180 mg) as crystals (from methanol). M.p. 106–108°, $[\alpha]_D^{22}$ $-55.2°$ (c 1.05). $C_{30}H_{50}O_2$: $M^{+\cdot}$ at m/z 442. ^1H NMR: m, δ 3.91 and 5.42 ppm (H-7 and H-15 respectively).

7α-Hydroxy-14α,15α-epoxy-apotirucalla-3-one **14**

The apo-compound **13** (63 mg) was epoxidised as described for **11** to give the epoxide **14** as prisms (from hexane–ether). M.p. 124–127°, $[\alpha]_D^{22}$ $-5.6°$. $C_{30}H_{50}O_3$: $M^{+\cdot}$ at m/z 458. ^1H NMR: m, δ 3.65 and 3.45 ppm (H-7 and H-15 respectively).

Δ^{14}-Apotirucalla-3,7-dione **15**

The apo-compound **13** (110 mg) was dissolved in acetone and cooled to 0°. Jones reagent was added dropwise until in an excess. The mixture was worked up in the usual way to give the diketone **15**, m.p. 103–105°, $[\alpha]_D^{22}$ $-134.4°$. $C_{30}H_{48}O_2$: $M^{+\cdot}$ at m/z 440. ^1H NMR: m, δ 5.75 ppm (H-15).

14,15-*Epoxy-apotirucalla-3,7-dione* **16**

(a) The diketo - apo - compound **15** (31 mg) was epoxidised as described for **11** to give the epoxide **16**. M.p. 156–159°, $[\alpha]_D^{22}$ $-8.9°$. $C_{30}H_{48}O_3$: $M^{+\cdot}$ at m/z 456. ^1H NMR: m, δ 3.90 (H-15). (b) Compound **14** (5 mg) was oxidised by Jones reagent as described for **15**. Working up in the usual manner gave a crystalline compound identical with the epoxide **16** obtained in a) (identity of m.p., MS and R_f on tlc in 3 solvent systems).

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