538. *The* Euphorbia *Resins. Part II. The Isolation of Taraxasterol and a New Triterpene, Tirucallol, from* E. tirucalli.

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Chromatographic separation of the resin from *E. tirucalli* gave euphol, previously reported by McDonald, Warren, and Williams (this vol., p. S 155), taraxasterol (characterised by its derivatives including *taraxasterone oxime*), and a new triterpene, *tirucallol*, $C_{30}H_{50}O$. Catalytic hydrogenation of *tirucallyl acetate* gave *dihydrotirucallyl acetate*, and thence *dihydrotirucallol*, both of which gave a yellow colour in tetranitromethane. *Tirucallyl benzoate*, oxidised with osmic acid and then hydrolysed, gave *dihydroxydihydrotirucallol*, which with lead tetra-acetate yielded acetone. Tirucallol thus contains an *iso*propylidene group and an inert ethylenic linkage, and is a tetracyclic triterpene alcohol.

Chromatographic analysis of the resin from *E. tirucalli* revealed very small quantities of two triterpenes in addition to euphol previously reported by McDonald, Warren, and Williams (this vol., p. S 155). These compounds were more strongly adsorbed than euphol, but adsorption on alumina of different activities was unsatisfactory in that the concentration of the eluant remained constant and the fractions showed no conspicuous difference in melting points until after crystallisation. The crude resin seemed to contain material which inhibited separation, and even euphol was obtained pure direct from the column only by use of the mixture obtained from the hydrolysis of the once-crystallised acetylated starting material. In the initial experiments a large column was first used to effect partial removal of the large quantities of euphol, the column was then deactivated, and the remainder of the material washed out and rechromatographed through a smaller column, light petroleum then removing the residual euphol. A mixture of benzene and light petroleum gave first a new triterpene, *tirucallol*, $C_{30}H_{50}O$, m. p. 133—134·5°, and then the third component, $C_{30}H_{50}O$,MeOH or $C_{30}H_{50}O$,H_2O, m. p. 226—227°, which after sublimation at 180°/0·01 mm. had the same melting point but gave analytical values corresponding to $C_{30}H_{50}O$.

A more satisfactory method used later was to percolate a light petroleum solution of the crude resin through a smaller column until all the euphol and tirucallol had been removed and then to elute the third component with light petroleum–benzene. The mixture of euphol and tirucallol was then acetylated and crystallised three times from ethanol to give pure euphyl acetate. The first mother-liquor, when stored, gave nodular crystals of a mixture whence rapid partial dissolution in warm ethanol left an amorphous solid which crystallised from acetone to give pure *tirucallyl acetate*, $C_{32}H_{52}O_2$; this was identical with the acetate of tirucallol obtained direct from the column, and gave on hydrolysis tirucallol which was further characterised as its *benzoate*. The formula was assigned after molecular-weight determinations by the method of Sandquist and Gorton (*Ber.*, 1930, **63**, 1025). Catalytic hydrogenation of the acetate revealed the presence of one active double bond and gave *dihydrotirucallyl acetate*, $C_{32}H_{54}O_4$, readily hydrolysed to *dihydrotirucallol*. The dihydro-compound gave a light-yellow colour in tetranitromethane, indicative of an ethylenic linkage, so that tirucallol contains two double bonds and is tetracyclic.

To determine the nature of the active ethylenic linkage, tirucallyl benzoate was treated with osmic acid and hydrolysed to give *dihydroxydihydrotirucallol*, which was then oxidised with lead tetra-acetate; acetone was obtained. An *iso*propylidene grouping thus exists in tirucallol, which in all the groupings determined is similar to euphol (cf. Newbold and Spring, *J.*, 1944, 251; McDonald, Warren, and Williams, *loc. cit.*).

The third component readily gave an acetate, $C_{32}H_{52}O_2$, m. p. 255—256°, which gave on hydrolysis an alcohol, m. p. 227—228°, undepressed on admixture with the original alcohol. The suggestion made by Dr. Hs. K. Krusi of these laboratories that the substance was taraxasterol was borne out by the properties of its derivatives (Burrows and Simpson, *J.*, 1938, 2042; Lardelli and Jeger, *Helv. Chim. Acta*, 1948, **31**, 813) (see table) and, after sublimation at 180° in a high vacuum, by analysis.

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	M. p.	$[\alpha]_D$.	M. p. (uncorr.).	$[\alpha]_D$.	M. p.	$[\alpha]_D$.
Taraxasterol	226 —227°	+108°	221—222°	+ 95·9°	225—226°	+ 91°
Taraxasteryl acetate	255 —256	+ 98	251—252	+100·5	256—257°	+100
Taraxasteryl benzoate	242 —244	+110	240—241	+106·8	—	—
Taraxasterone	184 —184·5	+147	175—176	+109·5	182—183	+110
Taraxasteroxime	266·5—267·5	+ 96	—	—	—	—
Dihydrotaraxasterol	222 —223	+ 11	—	—	218—220°	+ 11
Dihydrotaraxasteryl acetate	264 —265	+ 25	—	—	262—263	+ 23

It is of interest that taraxasterol has previously been obtained only from the *Compositæ* and isolated pure only by way of its acetate, whereas we have now obtained it pure direct from the material eluted from the column. In view of the isolation of ψ-taraxastanediol as a precursor of ψ-taraxasterol by Morice and Simpson (*J.*, 1938, 2046; 1941, 181) it seemed possible that our material, highly adsorbed on, and obtained directly from, the column, might be the dihydric precursor which resisted drying at 100° in a high vacuum but was dehydrated by sublimation or by acetylation. The material obtained, however, gave a yellow colour in tetranitromethane and was smoothly reduced to dihydrotaraxasterol. Power and Browning (*J.*, 1912, **101**, 2423) who first obtained taraxasterol record a hydrate, $C_{29}H_{48}O,2\frac{1}{2}H_2O$, water being determined by the loss of weight on drying at 125°, although, since they crystallise from ethanol, $C_{30}H_{50}O,C_2H_6O$ would equally well fit their data and would be in conformity with our solvate $C_{30}H_{50}O,CH_4O$ obtained after crystallisation from methanol.

The previously well-characterised triterpenes isolated from the *Euphorbia* resins have been tetracyclic monohydroxy-compounds, $C_{30}H_{50}O$, and the present isolation of the pentacyclic taraxasterol belonging to the lupeol group is of significance for assignment of ring structure.

Experimental.

All m. p.s are corrected.

Separation of the Components in the Resin from E. tirucalli *L.*—(*a*) Powdered resin (25 g.) was dissolved in light petroleum (350 ml.; b. p. 35—60°), and after 3 days the insoluble material (3 g.) was filtered off. The solution was percolated through a column of activated alumina (630 g.; 50 × 3·5 cm.) and analysed into 50 fractions of 300 ml. each. These, and the materials obtained from them, may be grouped as follows: (i) 1500 ml., trace of yellow gum; (ii) 100 ml., 1·1 g., m. p. 70—80° (from acetone, m. p. 70—80°); (iii) 1200 ml., 1·54 g., m. p. 70—80° (from acetone, m. p. 93—96°); (iv) 4200 ml., 3·78 g., m. p. 80—90° (from acetone, m. p. 105—112°); (v) 600 ml., 0·52 g., m. p. 107° (from acetone, m. p. 112°); and (vi) 600 ml., 0·42 g., m. p. 79—81°.

The column was then eluted with benzene–ethanol and the material rechromatographed through alumina (200 g.); the eluates and the materials from them may be grouped as follows: (vii) light petroleum (b. p. 60—80°) (5130 ml.), 7·51 g., m. p. 96—102°; (viii) 500 ml., 2·15 g., m. p. 125—128°; (ix) 980 ml., 2·15 g., m. p. 90—101°; (x) 520 ml., 0·8 g., m. p. 190—193°; and (xi) 530 ml., 0·96 g., m. p. 219—224°. Fractions (viii)—(xi) were obtained by benzene–light petroleum, and all the m. p.s refer to material recrystallised from methanol.

(*b*) Powdered resin (100 g.) in light petroleum (2 l.; b. p. 50—70°) was filtered after 2 weeks from the insoluble residue (12 g.), and the solution concentrated under reduced pressure to 500 ml. and filtered through a column of alumina (300 g.). The column was then developed with (i) light petroleum (6 l.) and (ii) light petroleum–benzene (1 : 1; 1500 ml.).

Tirucallol.—The rechromatography of fraction (*a*; ix) with light petroleum (b. p. 50—70°) through alumina (9 × 1 cm.) and crystallisation from methanol gave a solid, m. p. 125—127°, which combined with fraction (*a*; viii) and repeatedly crystallised gave *tirucallol* as long needles, $[\alpha]_D^{20}$ +4·5° (*c*, 2 in benzene), m. p. 133—134·5°, undepressed on admixture with a specimen obtained by hydrolysis of the acetate (Found: C, 84·1; H, 12·0. $C_{30}H_{50}O$ requires C, 84·45; H, 11·8%).

Tirucallyl Acetate.—(*a*) Tirucallol (100 mg., 1 mol.), acetic anhydride (500 mg., 20 mols.), and pyridine (500 mg., 25 mols.) were heated at 100° for 90 minutes and poured into water. The solid product crystallised from acetone, from which *tirucallyl acetate* separated in long, stout, brittle needles, m. p. 163·5°, $[\alpha]_D^{20}$ −16·7° (*c*, 1 in benzene) (Found: C, 81·7; H, 11·1. $C_{32}H_{52}O_2$ requires C, 82·0; H, 11·2%).

(*b*) The mother-liquor from the first crystallisation of crude euphyl acetate, obtained by acetylation of fraction (*b*; 1), was allowed to evaporate, to deposit a nodular amorphous solid. This was gently warmed with vigorous shaking, and the more insoluble portion quickly filtered off and crystallised thrice from acetone, to give tirucallyl acetate as long brittle needles, m. p. 162·5—163·5°, undepressed on admixture with that recorded in the preceding paragraph.

Tirucallyl Benzoate.—The alcohol (140 mg.), pyridine (1 ml.), and benzoyl chloride (1 ml.) at 100° gave a colourless substance which was purified by running an ethereal solution of it through a column of alumina and then crystallised from acetone–methanol, to give *tirucallyl benzoate* in flat plates, m. p 149—151°, $[\alpha]_D^{20}$ +10·8° (*c*, 1 in benzene) (Found: C, 84·0; H, 10·6. $C_{37}H_{54}O_2$ requires C, 83·7; H, 10·25%).

Dihydrotirucallol.—Tirucallyl acetate (303 and 340 mg.) in ethyl acetate (50 ml.) was hydrogenated in the presence of Adams's catalyst (100 mg.), and the absorption was complete after 140 minutes (a blank determination was carried out under similar conditions) (observed: 15·4 and 14·0 ml. at N.T.P. Calc. for $C_{32}H_{52}O_2$: 1 mol. = 14·5 and 16·3 ml., respectively). After 2 hours the product was isolated and crystallised from ethanol, to give *dihydrotirucallyl acetate* as long flat needles, m. p. 147—149°, $[\alpha]_D^{20}$ −11·6° (*c*, 1 in benzene) (Found: C, 81·7, 81·6; H, 11·4, 11·5. $C_{32}H_{54}O_2$ requires C, 81·6; H, 11·6%), which gave a yellow colour in tetranitromethane. The acetate, hydrolysed with 3% alcoholic potash (20 ml.) for 4 hours, gave a solid which, crystallised twice from acetone and then from methanol, gave *dihydrotirucallol* as needles, m. p. 150—151°, $[\alpha]_D^{20}$ +3° (*c*, 1 in benzene) (Found: C, 83·9, 84·2; H, 12·0, 12·2. $C_{30}H_{52}O$ requires C, 84·0; H, 12·2%).

Dihydroxydihydrotirucallol.—Tirucallyl benzoate (500 mg.) and osmic acid (245 mg.) in ether (50 mg.) were set aside for 5 days. The solution was then evaporated, and the residue added to a solution of potassium hydroxide (1 g.) and mannitol (1·6 g.) in ethanol–benzene (40 ml.) and boiled under reflux for 5 hours. The solvent was removed under reduced pressure, the residue extracted with ether, and the solution percolated through activated alumina (20 g.). Ether (300 ml.) eluted nothing from the column,

whilst alcohol (100 ml.) gave a crystalline solid (400 mg.) which, crystallised from ether–light petroleum (b. p. 50—60°), gave *dihydroxydihydrotirucallol* in laminæ, m. p. 172—173°, $[\alpha]_D^{20}$ −26·4° (*c*, 0·2 in benzene) (Found : C, 75·95, 75·9; H, 11·45, 11·3. $C_{30}H_{52}O_3$ requires C, 78·2; H, 11·4. $C_{30}H_{52}O_3,H_2O$ requires C, 75·3; H, 11·4%).

Oxidation with Lead Tetra-acetate.—Dihydroxydihydrotirucallol (192 mg.) in acetic acid (11 ml.) was added to lead tetra-acetate (260 mg.) in acetic acid (11 ml.) and set aside for 11 hours at 20°. Water was added and 6 ml. of the solution were distilled off. To 2 ml. of the distillate were added 2 : 4-dinitrophenylhydrazine (150 mg.), sulphuric acid (0·5 ml.), and ethanol (2 ml.), and the whole was set aside for 12 hours. Yellow needles, m. p. 124—125°, undepressed by an authentic specimen of acetone 2 : 4-dinitrophenylhydrazone (m. p. 124—125°), were obtained. Complete removal of the solvent gave a white solid which showed aldehydic properties but could not be separated pure or oxidised to an acid.

Taraxasterol.—Material obtained from fractions (*a*; x and xi) and from (*b*; ii) was crystallised 3 times from methanol, to give taraxasterol as colourless needles, m. p. 226—227°, $[\alpha]_D^{20}$ +108° (*c*, 1 in benzene) (Found : C, 81·6; H, 11·6. $C_{30}H_{50}O,CH_3{\cdot}OH$ requires C, 81·1; H, 11·9%). Sublimation of taraxasterol at 180°/0·01 mm. gave fine needles, m. p. 226—227° (Found : C, 84·4; H, 11·8. Calc. for $C_{30}H_{50}O$: C, 84·45; H, 11·8%). Acetylation of the alcohol (100 mg.) with acetic anhydride (2 ml.) in pyridine (3 ml.) at room temperature for 72 hours and crystallisation of the product from acetone gave taraxasteryl acetate as pearly plates, m. p. 255—256°, $[\alpha]_D^{20}$ +98° (*c*, 1 in benzene), unchanged after several crystallisations (Found : C, 81·45; H, 11·1%; *M*, 465 ± 1. Calc. for $C_{32}H_{52}O_2$: C, 82·0; H, 11·2%; *M*, 469). Hydrolysis with alcoholic potash gave taraxasterol. Benzoylation of the alcohol (100 mg.) in pyridine (2 ml.) with benzoyl chloride at 100° for 3 hours and pouring the mixture into water gave a sticky red mass. The ethereal extract was washed with sodium carbonate solution, dried, and percolated through a column of alumina (6 × 1 cm.) and a layer of charcoal to give a colourless solution. Crystallisation of the extract from methanol–acetone gave taraxasteryl benzoate as long, colourless needles, m. p. 242—244°, $[\alpha]_D^{20}$ +110° (*c*, 1 in benzene), unchanged after several crystallisations (Found : C, 83·05, 83·3, 83·4; H, 10·3, 10·2, 10·4. Calc. for $C_{37}H_{54}O_2$: C, 83·7; H, 10·25%).

Taraxasterone.—Copper oxide (100 mg.) was added during 5 minutes to taraxasterol (500 mg.) at 300° in an atmosphere of carbon dioxide, and the temperature maintained for 20 minutes. The product, in light petroleum (b. p. 50—70°), was percolated through alumina and then crystallised 3 times from acetone, to give taraxasterone as brittle, stellate crystals, m. p. 184—184·5°, $[\alpha]_D^{20}$ +147° (*c*, 1 in chloroform) (Found : C, 84·4; H, 11·0. Calc. for $C_{30}H_{48}O$: C, 84·8; H, 11·4%). The *oxime* crystallised from ethanol in stout, brittle needles, m. p. 266·5—267·5°, $[\alpha]_D^{20}$ +96° (*c*, 1 in chloroform) (Found : C, 81·9; H, 11·2. $C_{30}H_{49}ON$ requires C, 81·9; H, 11·2%).

Dihydrotaraxasterol.—Taraxasterol (302 mg.) in ethyl acetate–acetic acid (1 : 1; 100 ml.) with platinum oxide (50 mg.) was hydrogenated as described for the tirucallyl acetate. The hydrogen absorption was complete in 1 hour (observed : 14·7 ml. at N.T.P. Calc. for $C_{30}H_{50}O,CH_3{\cdot}OH$: 1 mol. = 15·2 ml.). The product crystallised from chloroform–methanol to give dihydrotaraxasterol as needles, m. p. 222—223°, $[\alpha]_D^{20}$ +11° (*c*, 1 in chloroform), giving no colour in tetranitromethane (Found : C, 83·5; H, 12·1. Calc. for $C_{30}H_{52}O$: C, 84·0; H, 12·2%). Acetylation of the alcohol (200 mg.) with acetic anhydride in pyridine and crystallisation of the product 3 times from ethanol gave dihydrotaraxasteryl acetate as very long needles, m. p. 264—265°, $[\alpha]_D^{20}$ +25 (*c*, 1 in chloroform), which gave no colour in tetranitromethane (Found : C, 81·4; H, 11·55. Calc. for $C_{32}H_{54}O_2$: C, 81·6; H, 11·6).

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