65. *The Isolation of Euphol and α-Euphorbol from Euphorbium.*

By G. T. Newbold and F. S. Spring.

Two crystalline monohydric alcohols have been isolated by the chromatographic method from euphorbone, an amorphous solid obtained from euphorbium. One of these is identical with α-euphorbol previously isolated in very small yield from the same source by Bauer and Schröder (*Arch. Pharm.*, 1931, **269**, 209). α-Euphorbol, which has been characterised by the preparation of esters, contains at least two double bonds. The second component, *euphol*, probably has the structure $C_{30}H_{50}O$. It is a monohydric alcohol containing two double bonds, one of which is relatively inert. It is tetracyclic and may belong to the triterpene alcohol group.

Extraction of euphorbium, the resinified latex of *Euphorbia spp.*, with light petroleum gives in fair yield a solid product, " euphorbone " (see Bauer and Schenkel, *Arch. Pharm.*, 1928, **266**, 633, where the earlier literature is cited and reviewed). Euphorbone is not homogeneous, and from it, by a tedious process of fractional crystallisation, two alcohols, α-euphorbol, $C_{26}H_{46}O$, m. p. 127—128°, and β-euphorbol, $C_{31}H_{52}O$, m. p. 89—90°, have been isolated (Bauer and Schenkel, *loc. cit.*; Bauer and Schröder, *Arch. Pharm.*, 1931, **269**, 209). The isolation of α-euphorbol from euphorbone (60 mg. from 750 g. of euphorbium) has also been achieved by Schmidt and

Zacherl (*Monatsh.*, 1931, **57**, 177). Although Müller (*J. pr. Chem.*, 1929, **121**, 97) isolated two alcohols from euphorbone, to which the names vitorbol, m. p. 120—125°, and novorbol, m. p. 123·5—124·5°, were given, Bauer and Schröder (*loc. cit.*) conclude that vitorbol is impure α-euphorbol and that novorbol is not homogeneous.

We have isolated two crystalline monohydric alcohols, in good yield and high purity, from euphorbium. Extraction of the drug with acetone gives an amorphous solid which corresponds closely in its properties to euphorbone. Filtration of a solution of this amorphous solid in light petroleum through a column of activated alumina, followed by washing with the same solvent, gives *euphol*. Subsequent washing of the column with benzene gives a second component, which is almost certainly identical with α-euphorbol.

Euphol, m. p. 116°, $[\alpha]_D$ +32°, which forms needles from acetone, is readily esterified to yield *euphyl acetate*, m. p. 109°, $[\alpha]_D$ +41°, and *euphyl benzoate*, m. p. 137—139°. Analyses of the alcohol and its esters give values in good agreement with the formula $C_{30}H_{50}O$ for the parent alcohol; however, it is to be noted that the analytical values do not exclude a number of near homologues (each of which has the general formula $C_nH_{2n-10}O$). Euphol is diethenoid; it gives a yellow coloration with the tetranitromethane reagent and its acetate absorbs two oxygen atoms from an excess of monoperphthalic acid. Of the two ethylenic linkages in euphol, one is unreactive. Euphyl acetate absorbs one mole of bromine to yield *euphyl acetate dibromide*, m. p. 138·5—139·5°, which gives a yellow coloration with the tetranitromethane reagent. Catalytic hydrogenation of euphyl acetate yields *dihydroeuphyl acetate*, m. p. 123·5—124°, which, like euphyl acetate, gives a yellow coloration with tetranitromethane. Dihydroeuphyl acetate absorbs one atom of oxygen from an excess of monoperphthalic acid, and on hydrolysis yields *dihydroeuphol*, m. p. 120°, further characterised as its *benzoate*, m. p. 160—161°. The two double bonds of euphol are not conjugated, since its acetate does not exhibit selective absorption of high intensity above 2200 A.

The homogeneity of euphol has been confirmed by conversion into the acetate, m. p. 109°, and thence into euphyl acetate dibromide, m. p. 138·5—139·5°; debromination of the last compound with zinc dust and acetic acid gives euphyl acetate, m. p. 109°, which is identical in crystalline form, melting point, and solubility with the original acetate. Hydrolysis of euphyl acetate gives euphol, m. p. 116°, again identical with the original specimen.

Although the analytical data obtained for euphol, dihydroeuphol and their esters do not exclude a number of close homologues of $C_{30}H_{50}O$ as possible formulæ for the parent alcohol, analysis of euphyl acetate dibromide appears to limit the possibilities to either $C_{30}H_{50}O$ or $C_{29}H_{48}O$. The amount of euphol which has been available has not been sufficient to allow a determination of the molecular weight by the reliable method of Sandqvist and Bengtsson. Since euphol is diethenoid and has the general formula $C_nH_{2n-9}OH$, it must be tetracyclic. It does not, however, appear to belong to the normal (3-β-hydroxy) sterol group, since it does not give an insoluble digitonide under conditions which lead to the quantitative precipitation of cholesterol. The ease with which euphol is washed from alumina with light petroleum is remarkable, and is in marked contrast to the tenacity with which alumina holds alcohols of the sterol and triterpene groups; this property of euphol probably has a constitutional significance.

The main benzene fraction from the chromatogram readily gave α-euphorbol. It was characterised as its *acetate*, m. p. 124—125°, which, like the parent alcohol, is optically inactive, and by the preparation of *α-euphorbyl benzoate*, m. p. 133—135°. α-Euphorbol gives a yellow coloration with the tetranitromethane reagent, and its acetate absorbs one mole of hydrogen to give dihydro-α-euphorbyl acetate, m. p. 133—135°, which still gives an unsaturation test with tetranitromethane. Hydrolysis of the dihydro-acetate yields dihydro-α-euphorbol, further characterised as its *benzoate*. A comparion of corresponding derivatives of the α-euphorbol prepared by Bauer and co-workers and by us shows that the two preparations are identical:

	Bauer.	This paper.
α-Euphorbol	M. p. 127—128°	M. p. 126—127°
	Optically inactive	$[\alpha]_D \pm 0°$
Dihydro-α-euphorbol	M. p. 133°	M. p. 132·5—134·5°
Dihydro-α-euphorbyl acetate	M. p. 129°	M. p. 133—135°
	—	$[\alpha]_D$ − 15°

The analytical values for carbon recorded by Bauer and Schenkel and by Bauer and Schröder are consistently lower (about 1%) than ours; these authors postulate the formula $C_{26}H_{46}O$ for α-euphorbol. The analytical data obtained on our derivatives are in good consistent agreement with the formula $C_{30}H_{50}O$ for the parent alcohol, but, as in the case of euphol, they do not exclude several near homologues of the general form $C_nH_{2n-10}O$. α-Euphorbyl acetate gives a *dibromide*, m. p. 169—171°, analysis of which appears to be more in agreement with the formula $C_{29}H_{48}O$ for the parent alcohol than with $C_{30}H_{50}O$, but a final decision on this point must be left until larger quantities of α-euphorbol are available.

In agreement with Bauer and Schenkel, we find that α-euphorbol does not give an insoluble digitonide. Furthermore, α-euphorbyl acetate cannot contain a conjugated diene system, since it does not exhibit selective light absorption of any appreciable intensity above 2200 A.

Both α-euphorbol and euphol give deep red colorations with the Liebermann–Burchard reagent, the solutions showing a green fluorescence which is reminiscent of that produced by the tetracyclic triterpene alcohol basseol (Heilbron, Moffet, and Spring, J., 1934, 1583). Experiments are in progress to examine the possible relationship of euphol and α-euphorbol to the triterpene alcohols.

Experimental.

Finely powdered euphorbium resin (500 g.) was extracted with boiling acetone (1 l.) for 2 hours. The orange-coloured liquid was filtered and kept for 48 hours. The white waxy solid (euphorbone) (37 g.), m. p. 105—110°, was collected; it was very soluble in methyl alcohol, acetone, and ethyl acetate, and, although long fine needles separated from a solution in the last solvent, it was not possible to effect a purification by this means, since crystallisation did not set in until the solution was extremely concentrated. Euphorbone separated in long fine needles, m. p. 65—70°, from light petroleum (b. p. 60—80°). The m. p. of this crystalline form was not altered by further crystallisation from the same solvent, and when attempts were made to recrystallise it from acetone, methyl alcohol or alcohol, it separated as an amorphous powder, m. p. between 102° and 110°. When it was heated at 40° in a vacuum, the light petroleum crystallisate lost both solvent of crystallisation and its crystalline form, the m. p. of the heat-treated specimen being 105—107°. This behaviour was observed by Tschirch and Paul (*Arch. Pharm.*, 1905, **243**, 273) and by Schmidt and Zacherl (*loc. cit.*). Euphorbone contains only a trace of saponifiable matter, and a saponified specimen showed the same behaviour towards solvents as the original euphorbone. Attempts were next made to purify euphorbone by acetylation, but again the product separated as an amorphous powder from acetone, alcohol, and ethyl acetate. Although this acetylated product readily absorbed bromine, attempts to isolate a crystalline bromide were equally unsuccessful.

Euphorbone (20 g.) was extracted (Soxhlet) with light petroleum (b. p. 60—80°; 1 l.) for 3 hours. A yellow rubber-like residue (2·5 g.) remained in the thimble. The solution was set aside at room temperature for 24 hours; a resinous deposit (*ca.* 1·0 g.) had then separated; after removal of the clear solution, the resin proved to be insoluble in light petroleum. The solution was filtered through a column of activated alumina (40 × 4 cm.), and the chromatogram washed with light petroleum (b. p. 60—80°), benzene, and ether to give the following fractions :

Fraction.	Solvent.		
I	Light petroleum	1000 c.c.	Trace
II	,, ,,	500 ,,	0·2 g.; colourless viscous oil
III	,, ,,	1000 ,,	3·5 g.; colourless crystalline solid
IV	,, ,,	1000 ,,	3·2 ,, ,, ,, ,,
V	,, ,,	1000 ,,	1·1 ,, ,, ,, ,,
VI	,, ,,	500 ,,	0·2 ,, ,, ,, ,,
VII	Benzene	500 ,,	4·2 ,, ,, ,, ,,
VIII	,,	500 ,,	0·5 ,, ,, ,, ,,
IX	Ether	500 ,,	0·7 ,, ,, ,, ,,

Fraction II could not be obtained crystalline from methyl alcohol, acetone and alcohol as solvents; it was extremely soluble in these solvents. Fraction III separated from acetone in rosettes of well-formed needles, m. p. 114—115°, which after one recrystallisation from the same solvent gave *euphol*, m. p. 116°, not altered by further recrystallisation, $[\alpha]_D^{19.5°}$ +32° (*l* = 1, *c* = 1·2 in chloroform). Fractions IV and V behaved in the same way, giving euphol as needles from acetone, m. p. 116°, undepressed with the specimen from fraction III (Found : C, 84·5, 84·6; H, 12·0, 12·1; active H, 0·27. $C_{30}H_{50}O$ requires C, 84·4; H, 11·8; 1 active H, 0·23%. $C_{29}H_{48}O$ requires C, 84·4; H, 11·7; 1 active H, 0·245%).

Euphyl acetate, obtained by heating the alcohol (1 part) with acetic anhydride (5 parts) and pyridine (5 parts) for 1½ hours on the steam-bath, separated from alcohol in needles, m. p. 109°, $[\alpha]_D^{22°}$ +41° (*l* = 1, *c* = 2·1 in chloroform), $[\alpha]_D^{19°}$ +26° (*l* = 0·5, *c* = 6·0 in pyridine) [Found : C, 82·1; H, 11·0; *M* (Rast), 449. $C_{32}H_{52}O_2$ requires C, 82·0; H, 11·2%; *M*, 469]. The purification of the acetate by crystallisation is much less wasteful of material than the purification of the alcohol. After 18 hours at 35°, the acetate (1 mol.) had absorbed 2·02 atoms of oxygen from an excess of monoperphthalic acid. An attempt to crystallise the product of the reaction was unsuccessful; it still formed a resin after adsorption on activated alumina, followed by elution with chloroform. *Euphyl benzoate*, prepared by heating a solution of euphol (350 mg.) in pyridine (2 c.c.) and benzoyl chloride (2 c.c.) for 2 hours on the steam-bath, crystallised from methanol–acetone in needles, m. p. 137—139°, $[\alpha]_D^{18·5°}$ +59° (*l* = 0·5, *c* = 1·0 in pyridine) (Found : C, 83·5; H, 10·6. $C_{37}H_{54}O_2$ requires C, 83·7; H, 10·3%).

Euphyl Acetate Dibromide.—Euphyl acetate (0·604 g.) was dissolved in ether (3 c.c.) and treated at room temperature with a solution of bromine in acetic acid (3%). The bromine was absorbed instantaneously; when 7·0 c.c. had been added, the solution contained free halogen and absorption ceased (calc. for 1 |=, 6·9 c.c.). The ether was removed under reduced pressure, and the solution set aside overnight. The crystalline product was recrystallised from acetone and then from alcohol, from which the *dibromide* separated in clusters of fine needles, m. p. 138·5—139·5°, $[\alpha]_D^{20°}$ +23·5° (*l* = 1, *c* = 1·2 in chloroform) [Found (for two different preparations) : C, 61·0, 60·9; H, 8·3, 8·6; Br, 25·5, 25·7. $C_{32}H_{52}O_2Br_2$ requires C, 61·1; H, 8·3; Br, 25·4%. $C_{31}H_{50}O_2Br_2$ requires C, 60·6; H, 8·2; Br, 26·0%]. The dibromide (350 mg.) was heated under reflux with zinc dust (3 g.), acetic acid (40 c.c.), and water (3 c.c.) for 6 hours. The hot solution was filtered, concentrated to half-bulk, and precipitated with water. The product, isolated by means of ether, was crystallised from acetone, giving euphyl acetate, m. p. 109°, not depressed by an authentic specimen, $[\alpha]_D^{19°}$ +23° (*l* = 0·5, *c* = 2·1 in pyridine).

Dihydroeuphol.—A solution of euphyl acetate (1·2 g.) in ethyl acetate–acetic acid (1 : 1; 150 c.c.) was shaken with hydrogen and platinum (from 350 mg. of PtO_2). The hydrogen absorption was rapid and practically complete after 15 minutes (observed, 60 c.c. at N.T.P.; calc. for $C_{32}H_{52}O_2$: 1 |=, 58 c.c.). After 2 hours the product was isolated and twice crystallised from alcohol, yielding *dihydroeuphyl acetate* in long needles, m. p. 123·5—124°, $[\alpha]_D^{19°}$ +34·5° (*l* = 1, *c* = 1·9 in chloroform) (Found : C, 81·5; H, 11·5. $C_{32}H_{54}O_2$ requires C, 81·6; H, 11·6%). The dihydro-acetate did not absorb bromine and it gave a yellow coloration with tetranitromethane in chloroform; it absorbed 1·08 atoms of oxygen from an excess of monoperphthalic acid (after 18 hours at 35°). Hydrolysis of the dihydro-acetate with 3% alcoholic potassium hydroxide gave *dihydroeuphol*, which formed felted needles from aqueous acetone, m. p. 120°, $[\alpha]_D^{18·5°}$ +34° (*l* = 1, *c* = 1·4 in chloroform) (Found : C, 84·2; H, 12·2. $C_{30}H_{52}O$ requires C, 84·0; H, 12·2%). *Dihydroeuphyl benzoate*, prepared from the alcohol by means of benzoyl chloride and pyridine, separated from acetone–methyl alcohol in blades, m. p. 160—161° (Found : C, 82·7; H, 10·3. $C_{37}H_{56}O_2$ requires C, 83·3; H, 10·6%).

α-Euphorbol.—Fraction VI from the chromatogram was crystallised from acetone. It proved to be a mixture, rosettes of fine needles separating together with more robust prismatic needles. The m. p. was indefinite (90—110°) and purification by crystallisation was not achieved. Fraction VII, however, readily crystallised from acetone to yield a homogeneous mass of hard needles, m. p. 126—127°, not altered by repeated crystallisation from the same solvent. *α-Euphorbol* also separated from methyl alcohol in needles, m. p. 126—127°, $[\alpha]_D^{17°}$ ±0° (*c* = 0·7, *l* = 1, in chloroform) (Found : C, 84·0; H, 11·8. $C_{30}H_{50}O$ requires C, 84·4; H, 11·8%). *α-Euphorbyl acetate*, obtained from the alcohol (1 part) by heating on the steam-bath with acetic anhydride (3 parts) and pyridine (3 parts), separated from acetone in needles, m. p. 124—125°. A mixture of equal parts of α-euphorbyl acetate and euphyl acetate (m. p. 109°) had m. p. 87—94°

and a mixture of equal parts of α-euphorbyl acetate and dihydroeuphyl acetate (m. p. 123—124°) had m. p. 93—98°. α-Euphorbyl acetate gives a deep yellow coloration with tetranitromethane in chloroform; $[\alpha]_D^{16.5°}$ ±0° ($l = 1$, $c = 1.3$ in chloroform) (Found: C, 81·8; H, 11·2. $C_{32}H_{52}O_2$ requires C, 82·0; H, 11·2%). *α-Euphorbyl benzoate*, prepared from the alcohol by means of benzoyl chloride and pyridine, separated from acetone–methyl alcohol in needles, m. p. 133—135°, $[\alpha]_D^{15°}$ +15° ($l = 0.5$, $c = 5.3$ in pyridine) (Found: C, 83·9; H, 10·5. $C_{37}H_{54}O_2$ requires C, 83·7; H, 10·3%). A mixture of equal parts of α-euphorbyl benzoate and euphyl benzoate (m. p. 137—139°) melted between 105° and 119°.

Dihydro-α-euphorbyl Acetate.—A solution of α-euphorbyl acetate (0·5 g.) in ethyl acetate (25 c.c.) was added to a suspension of platinum (from 200 mg. of PtO_2) in ethyl acetate (25 c.c.). After shaking with hydrogen for 6 hours, the old catalyst was removed, and fresh catalyst (from 100 mg. of PtO_2) added. Shaking in the presence of hydrogen was continued for 15 hours; hydrogen absorption (total, 25·4 c.c. at N.T.P.; calc. for $C_{32}H_{52}O_2$ 1 |=, 23·9 c.c.) had then ceased. The product was crystallised from acetone, yielding dihydro-α-euphorbyl acetate as small plates, m. p. 133—135°, $[\alpha]_D^{15°}$ −15° ($l = 0.5$, $c = 6.0$ in pyridine); the m. p. was not altered by recrystallisation. Dihydro-α-euphorbyl acetate gave a pale yellow coloration with tetranitromethane in chloroform (Found: C, 81·3; H, 11·5. Calc. for $C_{32}H_{54}O_2$: C, 81·6; H, 11·6%).

Dihydro-α-euphorbol was obtained from the acetate (150 mg.) by refluxing with alcoholic potassium hydroxide solution (20 c.c., 3%) for 2 hours. After three crystallisations from acetone, it formed needles, m. p. 132·5—134·5° (Found: C, 84·1; H, 11·8. Calc. for $C_{30}H_{52}O$: C, 84·0; H, 12·2%).

Dihydro-α-euphorbyl benzoate, prepared from the alcohol by means of benzoyl chloride and pyridine, separated from acetone–methyl alcohol in small prisms, m. p. 132—134° (Found: C, 82·7; H, 10·8. $C_{37}H_{56}O_2$ requires C, 83·4; H, 10·6%).

α-Euphorbyl Acetate Dibromide.—α-Euphorbyl acetate (100 mg.) in ether (2 c.c.) was treated with a solution of bromine in acetic acid (5%). Contrary to the case of euphyl acetate, bromine was only slowly absorbed. A total volume of 0·9 c.c. of bromine solution (1·3 moles) was therefore added, and the solution set aside at room temperature for 1 hour. After removal of ether at room temperature the mixture was freed from acetic acid by standing over potassium hydroxide in an evacuated desiccator. The solid residue was recrystallised five times from acetone, yielding *α-euphorbyl acetate dibromide* as plates, m. p. 169—171° (decomp.) (Found: C, 60·2, 60·2; H, 7·9, 8·3. $C_{32}H_{52}O_2Br_2$ requires C, 61·1; H, 8·3%. $C_{31}H_{50}O_2Br_2$ requires C, 60·6; H, 8·2%). The dibromide gave a light yellow colour with tetranitromethane in chloroform.

We are indebted to Mr. J. Green for help in the initial stages of this work.

THE UNIVERSITY, MANCHESTER. [*Received, January 13th*, 1944.]