

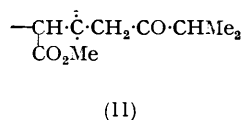
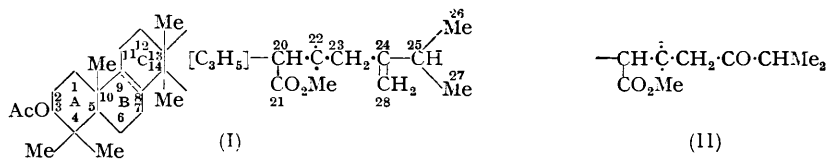
492. The Chemistry of Fungi. Part XIX.* The Structure of Eburicoic Acid.

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On treatment with acetic anhydride the hydrochloride of methyl *O*-acetyleburoic acid gave a mixture of the dienes, methyl 3-acetoxyeburico-7:24(28)-dien-21-oate (IV) and methyl 3-acetoxyeburico-7:23-dien-21-oate (V). Ozonolysis of (V) furnished methyl *isopropyl* ketone and an amorphous aldehyde (VIII) which was characterised by oxidation to the $\alpha\beta$ -unsaturated keto-ester (XII) and diketo-ester (XI). On oxidation with selenium dioxide *O*-acetyleburoic acid gave a lactone shown to be 3-acetoxy-23-hydroxyeburico-8:24(28)-dien-21-oic lactone (XIII; R = Ac).

The carboxyl group in eburicoic acid has been shown to be secondary and it is concluded that the side chain contains the residue $-\text{CH}(\text{CO}_2\text{H})\cdot\dot{\text{C}}\text{-CH}_2\cdot\text{C}(\text{:CH}_2)\cdot\text{CHMe}_2$. The transformation of eburicoic acid into lanost-8-ene confirms this and leads to a complete structure for eburicoic acid and its derivatives.

IN Parts XVI (*J.*, 1951, 2346), XVII (*J.*, 1953, 1830) and XVIII * evidence was advanced that eburicoic acid is a tetracyclic dienoic acid of type (I) containing the rings A, B, and C of the lanosterol cyclic system together with a vinylidene group. The experimental work now described shows that the acid contains the side chain $-\text{CH}(\text{CO}_2\text{H})\cdot\dot{\text{C}}\text{-CH}_2\cdot\text{C}(\text{:CH}_2)\cdot\text{CHMe}_2$. In this connection the steroid system of numbering the rings A, B, and C (Part XVIII) is now extended to cover this residue as in (I).

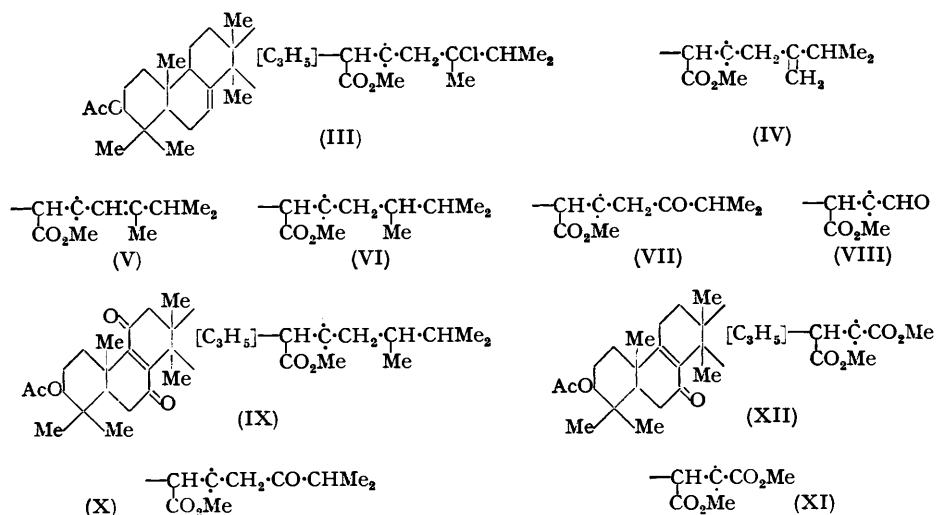


The infra-red absorption spectra of eburicoic acid and certain of its derivatives support the view that the reactive ethylenic double bond is present as a vinylidene group (cf. Part XVI). Thus the spectrum of eburicoic acid has a peak at 890 cm^{-1} characteristic of this group (Arnold, Koller, and Jeger, *Helv. Chim. Acta*, 1951, **34**, 555) whilst that of the ozonolysis product from methyl *O*-acetyleburoic acid, which is now regarded as methyl 3-acetoxy-24-keto-28-noreburico-8-en-21-oate, shows peaks at 1730 cm^{-1} , due to the acetoxy- and the carbomethoxy-group, and at 1715 cm^{-1} to the carbonyl group introduced by ozonolysis. Similarly, methyl 24-keto-28-noreburico-8-en-21-oate shows the same peaks. The observed infra-red frequency of the carbonyl absorption suggests that this group is

* Part XVIII, preceding paper.

exocyclic as in (II) or, less probably, in a six-membered ring system (cf. Grove and Willis, *J.*, 1951, 877). The possibility that the ozonolysis product, methyl 24-keto-28-noreburic-8-en-21-oate, was a methyl ketone was excluded by its failure to react with potassium hypiodite at 80°.

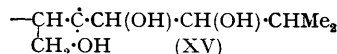
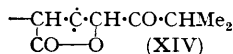
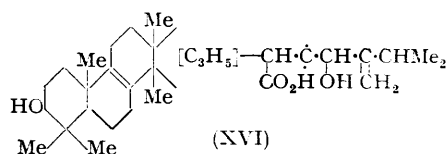
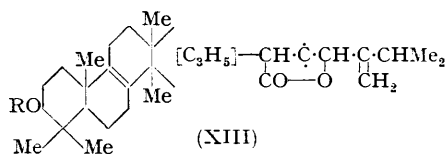
On treatment with hydrogen chloride in acetic acid methyl *O*-acetylebucricate gave an adduct in which the chlorine atom is labile. As expected, by analogy with methyl 3-acetoxyeburic-8-en-21-oate (methyl *O*-acetyldihydroeburicoate, Part XVII) this addition was accompanied by migration of the inert double bond from the Δ^6 - to the Δ^7 -position, thus giving (III). By the action of acetic anhydride on (III) hydrogen chloride was eliminated with the formation of two isomeric compounds which differ only in the position of the reactive double bond, *i.e.*, methyl 3-acetoxyeburico-7 : 24(28)-dien-21-oate (IV) and -7 : 23-dien-21-oate (V). Thus (IV) exhibited strong infra-red absorption at 823 and 890 cm^{-1} , in the region of olefinic C-H out-of-plane bending where the former frequency is due to the trisubstituted nuclear double bond (cf. Bladon, Fabian, Henbest, Koch, and Wood, *J.*, 1951, 2402) and the latter to the vinylidene group (cf. Arnold *et al.*, *loc. cit.*). On the other hand (V) had only one absorption peak at 823 cm^{-1} , in this region of the spectrum and, since there are two double bonds present, clearly either both must be trisubstituted or one tri- and the other tetra-substituted. The relation between (IV) and (V) was ultimately confirmed by their hydrogenation to the same dihydro-derivative (VI), identical with methyl 3-acetoxyeburic-7-en-21-oate (3-acetoxyeburic-7-enate in Part XVIII). Further, oxidation of (VI) obtained from either source gave the same diketone (IX), identical with methyl 3-acetoxy-7 : 11-diketoeburic-8-en-21-oate (3-acetoxy-7 : 11-diketoeburic-8-enate of Part XVII).



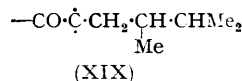
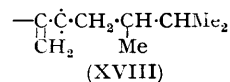
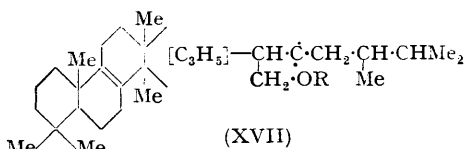
That (IV) containing a methylene group differed from (I) only with regard to the position of the inactive (nuclear) double bond was established by the ozonolysis of (IV) to formaldehyde and methyl 3-acetoxy-24-keto-28-noreburic-7-en-21-oate (VII) which on oxidation with chromic anhydride furnished methyl 3-acetoxy-7 : 11 : 24-triketo-28-noreburic-8-en-21-oate (X), identical with the oxidation product obtained from methyl 3-acetoxy-24-keto-28-noreburic-8-en-21-oate (II). On the other hand, the ozonolysis of (V) gave methyl isopropyl ketone and an amorphous aldehydic product (VIII) which on oxidation with chromic anhydride under comparatively mild conditions and subsequent esterification furnished the $\alpha\beta$ -unsaturated keto-ester (XII) whereas on more vigorous oxidation, followed by esterification of the product, (VIII) gave the yellow $\alpha\beta$ -unsaturated diketo-ester (XI). Thus the side chain of eburicic acid contains the residue $\text{Me}_2\text{CH}\cdot\text{C}(\text{CH}_2)\cdot\text{CH}_2^-$.

By the oxidation of *O*-acetylebucric acid with an equivalent of selenium dioxide a

neutral product was obtained which is regarded as the lactone (XIII; R = Ac). The presence of the lactone ring was established by conversion into the acid (XVI) and this, on being heated, gave the lactone (XIII; R = H) which, on acetylation, regenerated the parent compound (XIII; R = Ac). In agreement with this the lactone (XIII; R = Ac) exhibited strong infra-red absorption at 890, 1730, and 1779 cm^{-1} characteristic



respectively of a vinyl, an acetoxy-, and a lactone group where the lactone is probably of the γ -type (cf. Grove and Willis, *loc. cit.*). Further, on ozonolysis (XIII; R = Ac) gave formaldehyde and 3-acetoxy-23-hydroxy-24-keto-28-noreburic-8-en-21-oic lactone (XIV; R = Ac) which, on reduction with lithium aluminium hydride, furnished 28-noreburic-8-ene-3 : 21 : 23 : 24-tetraol (XV; R = H). With lead tetra-acetate this tetraol yielded isobutyraldehyde which must arise by scission between $\text{C}_{(23)}$ and $\text{C}_{(24)}$, thus showing that hydroxylation of *O*-acetyleburiac acid with selenium dioxide had taken place at $\text{C}_{(23)}$. Hence, on the view that the lactone ring present in (XIII; R = Ac) is five-membered, the carboxyl group in eburicoic acid is attached at $\text{C}_{(20)}$, *i.e.*, the side chain contains the residue $>\text{C}(\text{CO}_2\text{H})\cdot\dot{\text{C}}\cdot\text{CH}_2\cdot\text{C}(\text{:CH}_2)\cdot\text{CHMe}_2$.



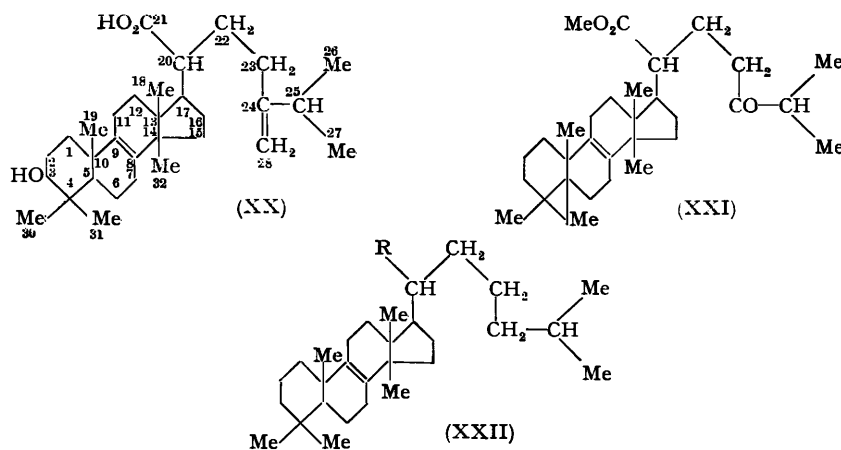
By the method which Arnold *et al.* (*loc. cit.*) employed in the case of elemadienolic acid this carboxyl group was shown to be secondary. The benzoate (XVII; R = Bz) of eburic-8-en-21-ol (eburicenol, Part XVI, *loc. cit.*) was pyrolysed in nitrogen, giving benzoic acid and eburico-8 : 20-diene (XVIII), the infra-red spectrum of which exhibited strong absorption at 887 cm^{-1} characteristic of a vinyl group. In agreement with this the ozonolysis of (XVIII) gave formaldehyde and 21-noreburic-8-en-20-one (XIX) having strong absorption in the infra-red at 1715 cm^{-1} characteristic of a non-conjugated exocyclic keto-group (cf. Grove and Willis, *loc. cit.*). Consequently, the foregoing expression for the side chain of eburicoic acid may be expanded to $\cdot\text{CH}(\text{CO}_2\text{H})\cdot\dot{\text{C}}\cdot\text{CH}_2\cdot\text{C}(\text{:CH}_2)\cdot\text{CHMe}_2$.

In view of the tetracyclic nature of eburicoic acid and its established relation to lanosterol with respect to rings A, B, and C, in conjunction with the partial structure now established for the side chain, it appeared that the C_3H_5 residue is present in a cyclopentane system as in lanosterol. Consequently, $\text{C}_{(22)}$ is present as a methylene group and, on the assumption that the side-chain is attached at $\text{C}_{(17)}$, the only difference between the carbon skeletons of eburicoic acid and lanosterol appeared to be the presence of the extra carbon atom $\text{C}_{(28)}$ in the side chain of the acid. Therefore, from the established structure of lanosterol (Curtis, Fridrichsons, and Mathieson, *Nature*, 1952, **170**, 321; Voser, Mijovic, Heusser, Jeger, and Ruzicka, *Helv. Chim. Acta*, 1952, **35**, 2414; Barnes, Barton, Fawcett, and Thomas, *J.*, 1953, 576) eburicoic acid may be represented by (XX). Since the position of the methylene group in the side chain of eburicoic acid has been established, the following conversion of methyl 24-keto-28-noreburic-8-en-21-oate (XXI) into lanost-8-ene (lanostene; Ruzicka, Rey, and Muhr; *Helv. Chim. Acta*, 1944, **27**, 472; Ruzicka, Denss, and Jeger, *ibid.*, 1945, **28**, 759; Dorée, McGhie, and Kurzer, *J.*, 1947, 1467) provides

conclusive evidence in support of this structure (XX) for eburicoic acid and further, in the absence of steric rearrangements during its conversion into (XXII; R = Me), eburicoic acid has the same configuration as lanosterol.

On reduction by the Wolff-Kishner method followed by esterification of the product, methyl 24-keto-28-noreburic-8-en-21-oate gave methyl 28-noreburic-8-en-21-oate (XXII; R = CO₂Me) which was reduced with lithium aluminium hydride to 28-noreburic-8-en-21-ol (XXII; R = CH₂-OH). Oxidised by the Oppenauer procedure, this alcohol furnished 28-noreburic-8-en-21-al (XXII; R = CHO), and by the Wolff-Kishner method this aldehyde gave 28-noreburic-8-ene (XXII; R = Me) which, by comparison of their melting points, specific rotations, and infra-red absorption spectra, was found to be identical with lanost-8-ene. It is of interest that, in respect of the carbon skeletons of their side chains, lanosterol bears the same relation to eburicoic acid as cholesterol to ergosterol.

With regard to the stereochemical relation of eburicoic acid and lanosterol, there is evidence that the configurations of the hydroxyl groups at C₍₉₎ are identical, *viz.*, the reduction of 3-ketoeburico-8:24(28)-dien-21-oic acid with sodium and alcohol gives eburicoic acid without the respective epimer (Part XVII), and dehydration of methyl 3-hydroxyeburico-8-en-21-oate with phosphorus pentachloride is accompanied by rearrangement of ring A (Part XVI) (cf. Barnes *et al.*, *loc. cit.*).



In the formation of methyl eburic-8-en-21-oate from eburicoic acid by the use of Oppenauer and Wolff-Kishner procedures (Part XVI) stereochemical rearrangement appeared not to occur because the same product was obtained on hydrogenation of methyl eburico-2:8-dien-21-oate formed by the dehydration of methyl 3-hydroxyeburico-8-en-21-oate with phosphoryl chloride (Part XVIII) (cf. the conversion of lanosterol into lanost-8-ene; Dorée *et al.*, *loc. cit.*; McGhie, Pradhan, and Cavalla, *J.*, 1952, 3176). Since in the formation of methyl 28-noreburic-8-en-21-oate (XXII; R = CO₂Me) from eburicoic acid the same methods are employed it seems unlikely that this transformation was accompanied by steric rearrangement. For the conversion of methyl 28-noreburic-8-en-21-oate (XXII; R = CO₂Me) into lanost-8-ene the same procedures have been employed and it seems unlikely, therefore, that stereochemical rearrangement of the cyclic system occurs. The possibility of inversion at C₍₂₀₎ during transformation of (XXII; R = CO₂Me) into (XXII; R = Me) cannot at present be excluded although this is regarded as unlikely.

The steroid numbering of the molecule is now extended to include ring D and the angular methyl groups attached at C₍₁₀₎ and C₍₁₃₎. In accordance with the Editorial Report on Nomenclature, Appendix C, Steroid Nomenclature, Rule 1 (*J.*, 1951, 3526), the number 29, which is reserved for a carbon atom attached at C₍₂₈₎, is omitted and the angular methyl groups attached at C₍₄₎ and C₍₁₄₎ are numbered 30, 31, and 32 respectively as in (XX), a system which was adopted after discussion with Professor E. R. H. Jones, F.R.S., and Dr. T. G. Halsall of the University of Manchester.

EXPERIMENTAL

Unless stated otherwise light petroleum employed had b. p. 60—80°, optical rotations were measured in chloroform at room temperature (18—22°), ultra-violet absorption spectra in 95% alcohol, and infra-red spectra in 20% carbon disulphide solutions.

Methyl 24-Keto-28-noreburic-8-en-21-oate (XXI).—A current of ozone and oxygen was led into a solution of methyl eburico-8 : 24(28)-dien-21-oate (methyleburicodienate, Part XVI, *loc. cit.*) (2.5 g.) in acetic acid (250 ml.) for 2.5 hr. After the dilution of the mixture with water (2 l.) the formaldehyde was distilled with steam and converted into the dimedone derivative (0.92 g., 56%). The non-volatile residue was collected and chromatographed on aluminium oxide (24 × 1.6 cm.) from light petroleum (50 ml.). After being washed with light petroleum (400 ml.) to remove amorphous material (0.56 g.), the column was extracted with more light petroleum (200 ml.) and then benzene-light petroleum (1 : 1; 800 ml.), giving *methyl 24-keto-28-noreburic-8-en-21-oate* which separated from methanol in needles (1.5 g.), m. p. 123—125°, $[\alpha]_D + 50^\circ$ (*c*, 3.0; *l* = 0.5 dm.) (Found : C, 79.4; H, 10.7. C₃₁H₅₀O₃ requires C, 79.1; H, 10.7%). The *oxime* formed prisms, m. p. 158—160° (decomp.), from alcohol (Found : N, 2.9. C₃₁H₅₁O₃N requires N, 2.9%).

Action of Hydrogen Chloride on Methyl O-Acetylburicoate.—A solution of this ester (10 g.) in acetic acid (600 ml.) kept at room temperature was slowly saturated with hydrogen chloride and then poured into ice-water (1 l.). On isolation the resulting *hydrochloride* (III) separated from methanol in needles (8 g.), m. p. 173° (decomp.), $[\alpha]_D + 33^\circ$ (*c*, 1.97) (Found : Cl, 6.2. C₃₄H₅₅O₄Cl requires Cl, 6.3%), which on treatment with dilute alcoholic silver nitrate instantaneously gave a precipitate of silver chloride.

A solution of this hydrochloride (2 g.) in acetic anhydride (25 ml.) was boiled for 10 hr. and diluted with water. The solid was crystallised twice from methanol, giving a fraction in needles (1.03 g.), m. p. 165—168°, which was adsorbed on aluminium oxide (100 g.; 24 × 2.2 cm.) from light petroleum. After being washed with the same solvent the column was extracted with acetone-light petroleum (1 : 99), giving *methyl 3-acetoxyeburico-7 : 24(28)-dien-21-oate* (IV) which separated from methanol in needles, m. p. 173—174°, $[\alpha]_D + 32^\circ$ (*c*, 2.66) (Found : C, 77.4; H, 10.1. C₃₄H₅₄O₄ requires C, 77.5; H, 10.3%). The methanolic liquors left on separation of the solid, m. p. 165—168°, were concentrated, giving a solid (0.74 g.), m. p. 125—135°. From this material *methyl 3-acetoxyeburico-7 : 23-dien-21-oate* (V) was isolated by the same chromatographic technique and, on crystallisation from methanol, formed fine needles, m. p. 153—154°, $[\alpha]_D + 32^\circ$ (*c*, 2.47; *l* = 0.5 dm.) (Found : C, 77.9; H, 10.4. C₃₄H₅₄O₄ requires C, 77.5; H, 10.3%).

Hydrogenation (1 mol. absorbed) of methyl 3-acetoxyeburico-7 : 24(28)-dien-21-oate (IV) in acetic acid, with a platinum catalyst, gave an almost theoretical yield of methyl 3-acetoxyeburico-7-en-21-oate (VI) which was purified by chromatography and then from alcohol, forming needles, m. p. 172—174°, identical with an authentic specimen (Part XVIII). This hydrogenation product (1 g.) was oxidised with chromic anhydride (0.8 g.) in 95% acetic acid (30 ml.) at 80° during 1 hr., the excess of chromic acid was destroyed with a little methanol, and the product was precipitated with water (350 ml.). A solution of this in benzene-light petroleum (1 : 1; 30 ml.) was poured on aluminium oxide (17 × 1.8 cm.) which was then washed with acetone-benzene (1 : 19; 100 ml.), giving methyl 3-acetoxy-7 : 11-diketoeburic-8-en-21-oate (IX) which separated from methanol in large yellow prisms (0.41 g.), m. p. 171—172°, $[\alpha]_D + 68^\circ$ (*c*, 2.07; *l* = 0.5 dm.), λ_{\max} , 270 m μ (log ϵ 3.92), identical with an authentic specimen (methyl *O*-acetyldihydrodiketoeburicoate, Part XVII).

Ozonolysis of methyl 3-acetoxyeburico-7 : 24(28)-dien-21-oate (1 g.) in acetic acid (100 ml.), and decomposition of the product with water (1 l.) gave formaldehyde, isolated as the dimedone derivative (0.33 g.), m. p. 189°, and *methyl 3-acetoxy-24-keto-28-noreburic-7-en-21-oate* (VII), which formed needles (0.78 g.), m. p. 169—170°, $[\alpha]_D + 45^\circ$ (*c*, 1.08), from methanol (Found : C, 75.1; H, 9.6. C₃₃H₅₀O₅ requires C, 74.9; H, 9.9%). On oxidation by chromic anhydride-acetic acid this compound furnished methyl 3-acetoxy-7 : 11 : 24-triketo-28-noreburic-8-en-21-oate (X) which separated from light petroleum in yellow needles, m. p. 194—195°, $[\alpha]_D + 75^\circ$ (*c*, 0.91; *l* = 0.5 dm.), λ_{\max} , 270 m μ (log ϵ 3.94) (Found : C, 71.5; H, 9.1. Calc. for C₃₃H₄₈O₇ : C, 71.3; H, 8.7%). This product was also prepared from methyl 3-acetoxyeburico-8 : 24(28)-dien-21-oate (methyl *O*-acetylburicoate) (I) by the same two-stage procedure and appeared to be identical with the ester which Lahey and Strasser (*J.*, 1951, 873) obtained by the vigorous oxidation of (I) with chromic anhydride.

Hydrogenation (1 mol. of hydrogen absorbed) of methyl 3-acetoxyeburico-7 : 23-dien-21-oate

(V) furnished methyl 3-acetoxyeburic-7-en-21-oate (VI) which had m. p. and mixed m. p. 172—174°, after purification by chromatography and crystallisation from methanol and on oxidation with chromic anhydride in acetic acid gave methyl 3-acetoxy-7 : 11-diketoeburic-8-en-21-oate (IX), m. p. and mixed m. p. 171—172°.

Ozonolysis of methyl 3-acetoxyeburico-7 : 23-dien-21-oate (1 g.) in acetic acid (100 ml.) and treatment of the solution with water (1 l.), followed by distillation in steam, gave a distillate which on treatment with 1% aqueous dimedone gave the formaldehyde derivative (0.08 g.), m. p. 189°. The filtrate from the solid was distilled and mixed with 1% aqueous 2 : 4-dinitrophenylhydrazine sulphate (50 ml.), giving the 2 : 4-dinitrophenylhydrazone (0.24 g.) of methyl isopropyl ketone which was purified by chromatography on aluminium oxide and then from alcohol, forming orange needles, m. p. 123—124°, λ_{\max} . 227, 361 m μ (log ϵ 4.30, 4.38), identical with an authentic specimen (Found : C, 49.8; H, 5.4; N, 21.2. Calc. for C₁₁H₁₄O₄N₄ : C, 49.6; H, 5.3; N, 21.0%).

The non-volatile ozonolysis product (0.93 g.), which had aldehydic properties, did not crystallise. A solution of the crude substance (0.9 g.) in acetic acid (20 ml.) was mixed with chromic anhydride (0.22 g.) in acetic acid (6.6 ml.) and kept at 40° for 1 hr. Next day the product was precipitated with water (200 ml.) and isolated along with a little acetic acid by means of ether. After the removal of the acetic acid in a vacuum at room temperature the solid was dissolved in ether, and the solution extracted with 2N-sodium hydroxide. The alkaline extracts were acidified and the acidic material esterified with ethereal diazomethane. A solution of the resulting ester in benzene-light petroleum (1 : 4; 5 ml.) was poured on aluminium oxide (12 × 0.8 cm.), and the column washed with benzene-light petroleum (9 : 1), giving the *keto-ester* (XII) which separated from aqueous methanol in colourless needles, m. p. 196—198°, $[\alpha]_D +14^\circ$ (*c*, 1.0; *l* = 0.5 dm.), λ_{\max} . 251 m μ (log ϵ 3.76) (Found : C, 69.8; H, 8.9. C₃₀H₄₄O₇ requires C, 69.7; H, 8.6%).

When the non-volatile solid (1.78 g.) was oxidised with chromic anhydride (1.95 g.) in 95% acetic acid (60 ml.) at 80° and the product isolated and esterified by the same procedure the $\alpha\beta$ -unsaturated *diketo-ester* (XI) was obtained. This was purified by chromatography on aluminium oxide from benzene-light petroleum and then crystallised from chloroform-light petroleum, forming yellow plates, m. p. 209—212°, $[\alpha]_D +71^\circ$ (*c*, 1.18; *l* = 0.5 dm.), λ_{\max} . 270 m μ (log ϵ 3.94) (Found : C, 67.8; H, 8.0. C₃₀H₄₂O₈ requires C, 67.9; H, 8.0%).

3-Acetoxy-23-hydroxyeburico-8 : 24(28)-dien-21-oic Lactone.—A solution of *O*-acetyleburiocic acid (9 g.) in acetic acid (135 ml.) containing selenium dioxide (1 g.) and water (4.5 ml.) was heated under reflux for 3 hr., filtered, and poured into water. The dried precipitate (8.9 g.), dissolved in benzene-light petroleum (1 : 4) (300 ml.), was poured on neutralised aluminium oxide (30 × 2.9 cm.; 200 g.), and the column extracted with benzene-light petroleum (1 : 4; 400 ml.), giving a white solid (4.5 g.) which, on crystallisation from alcohol, yielded *3-acetoxy-23-hydroxyeburico-8 : 24(28)-dien-21-oic lactone* (XIII; R = Ac) in plates (2.6 g.), m. p. 214—215°, $[\alpha]_D +49^\circ$ (*c*, 2.0) (Found : C, 77.3; H, 9.6. C₃₃H₅₀O₄ requires C, 77.6; H, 9.9%). A solution of this lactone (0.5 g.) in 10% alcoholic potassium hydroxide (20 ml.) was boiled for 1 hr., diluted with water (100 ml.), cooled to 0°, and acidified with acetic acid, giving *3 : 23-dihydroxyeburico-8 : 24(28)-dien-21-oic acid* (XVI) which formed slender needles, m. p. 216° (decomp.), $[\alpha]_D +49^\circ$ (*c*, 2.16 in EtOH; *l* = 0.5 dm.), from ethyl acetate (Found : C, 76.7; H, 10.2. C₃₁H₅₀O₄ requires C, 76.5; H, 10.4%). On being kept at 216° until effervescence had ceased this acid was quantitatively converted into *3 : 23-dihydroxyeburico-8 : 24(28)-dien-21-oic lactone* (XIII; R = H) which separated from methanol in slender needles, m. p. 181—182°, $[\alpha]_D +48^\circ$ (*c*, 1.62; *l* = 0.5 dm.) (Found : C, 79.5; H, 10.3. C₃₁H₄₈O₃ requires C, 79.4; H, 10.3%). On acetylation by acetic anhydride-pyridine this regenerated the acetate, m. p. and mixed m. p. 214—215°.

3-Acetoxy-23-hydroxy-24-keto-28-noreburic-8-en-21-oic Lactone (XIV; R = Ac).—Ozonolysis of *3-acetoxy-23-hydroxyeburico-8 : 24(28)-dien-21-oic lactone* (8 g. in batches of 1 g.) in acetic acid gave formaldehyde, isolated as the dimedone derivative (yield, 44%), and *3-acetoxy-23-hydroxy-24-keto-28-noreburic-8-en-21-oic lactone* (XIV; R = Ac) (7.4 g.) which was purified by filtration through aluminium oxide (32 × 1.8 cm.) from benzene-light petroleum (200 ml.) followed by crystallisation from benzene-alcohol (1 : 10), forming plates (4.9 g.), m. p. 233—235°, $[\alpha]_D +59^\circ$ (*c*, 2.05) (Found : C, 75.2; H, 9.5. C₃₂H₄₈O₅ requires C, 74.9; H, 9.4%). The *oxime* separated from methanol in needles, m. p. 231° (decomp.) (Found : N, 2.7. C₃₂H₄₈O₅N requires N, 2.7%).

Reduction of this lactone (1.5 g.) with lithium aluminium hydride (5 g.) in boiling ether for 10 hr., followed by treatment of the reaction mixture with ice and dilute sulphuric acid, gave

28-noreburic-8-ene-3:21:23:24-tetrol (XV; R = H) which was isolated with ether and crystallised from alcohol-ethyl acetate (1:20), forming slender needles (1.1 g.), m. p. 214—215.5°, $[\alpha]_D + 53^\circ$ (*c*, 2.0 in EtOH) (Found, in specimen sublimed in a high vacuum: C, 75.3; H, 10.8. $C_{30}H_{52}O_4$ requires C, 75.6; H, 11.0%). On acetylation by acetic anhydride-pyridine this gave the *tetra-acetate*, forming needles, m. p. 100°, $[\alpha]_D + 53^\circ$ (*c*, 2.37), from dilute alcohol (Found: C, 71.2; H, 9.1. $C_{38}H_{60}O_8$ requires C, 70.8; H, 9.4%).

Oxidation of this tetrol (0.4 g.) with lead tetra-acetate (0.37 g.) in acetic acid (9 ml.) at room temperature was complete after 4 hr. Dilution with water then gave an amorphous solid which reduced Fehling's solution, formed an amorphous 2:4-dinitrophenylhydrazone, and on oxidation with chromic anhydride in acetic acid yielded intractable material.

Distillation of the aqueous-acetic acid filtrate from the solid gave *isobutyraldehyde* which was isolated as the 2:4-dinitrophenylhydrazone, m. p. 181—182°, after purification from alcohol, identical with an authentic specimen (yield, 44%) (Found: N, 22.3. Calc. for $C_{10}H_{12}N_4O_4$: N, 22.2%).

21-Noreburic-8-en-20-one (XIX).—Eburic-8-en-21-ol (XVII; R = H) (eburicenol, Part XVI) (3.5 g.) was benzoylated in pyridine. The resulting *benzoate* (XVII; R = Bz) separated from methanol in needles (2.9 g.), m. p. 100—102°, $[\alpha]_D + 40^\circ$ (*c*, 2.0) (Found: C, 83.9; H, 10.7. $C_{38}H_{58}O_2$ requires C, 83.5; H, 10.7%). On being kept in nitrogen at 340—350° for 2 hr. this (2.7 g.) gave a sublimate of benzoic acid. A solution of the residue in a mixture of benzene (135 ml.) and 10% methanolic potassium hydroxide (225 ml.) was boiled for 1½ hr. to hydrolyse unchanged benzoate, and then poured into water. The mixture was extracted with ether, and the ethereal extracts were washed with 2*N*-sodium hydroxide, dried, and evaporated, leaving a colourless oil. A solution of this in light petroleum (50 ml.) was poured on aluminium oxide (24 × 1.6 cm.), and the column eluted with the same solvent. Evaporation of the eluate left *eburico-8:20-diene* (XVIII) which separated from acetone in plates (0.98 g.), m. p. 73—74°, $[\alpha]_D + 45^\circ$ (*c*, 2.01; *l* = 0.5 dm.) (Found: C, 87.6; H, 12.3. $C_{31}H_{52}$ requires C, 87.7; H, 12.3%). Subsequent elution of the aluminium oxide with acetone-benzene (1:20) gave eburic-8-en-21-ol (1.4 g.), m. p. and mixed m. p. 100—102°.

Ozonolysis of eburico-8:20-diene (0.42 g.) in acetic acid (100 ml.) gave formaldehyde, isolated as the dimedone derivative, m. p. 189° (yield, 31%), and 21-noreburic-8-en-20-one (XIX) which separated from methanol in needles (0.22 g.), m. p. 101—102°, $[\alpha]_D + 98^\circ$ (*c*, 2.98; *l* = 0.5 dm.) (Found: C, 84.8; H, 11.9. $C_{30}H_{50}O$ requires C, 84.5; H, 11.8%). The *oxime* separated from dilute alcohol in needles, m. p. 137° (Found: N, 3.2. $C_{30}H_{51}ON$ requires N, 3.2%).

Methyl 28-Noreburic-8-en-21-oate (XXII; R = CO₂Me).—A mixture of methyl 24-keto-28-noreburic-8-en-21-oate (XXI) (2.6 g.), 90% hydrazine hydrate (0.8 ml.), potassium hydroxide (1.2 g.), and diethylene glycol (8 ml.) was heated under reflux for 3 hr. and then at 195° for 5 hr. The cooled mixture was poured into 3*N*-hydrochloric acid (18 ml.), and the precipitate collected, washed, dried, and treated with ethereal diazomethane. A solution of the resulting product in light petroleum (50 ml.) was poured on a column of aluminium oxide (20 × 2 cm.), and this eluted successively with (*a*) light petroleum (120 ml.), (*b*) light petroleum (200 ml.), (*c*) benzene-light petroleum (1:10; 50 ml.) and (*d*) benzene-light petroleum (1:1; 50 ml.). Evaporation of the eluate (*a*) gave amorphous material, whilst (*b*), (*c*), and (*d*) yielded a crystalline product (1.28 g.) which on crystallisation from methanol gave *methyl 28-noreburic-8-en-21-oate* in needles, m. p. 107—108°, $[\alpha]_D + 48^\circ$ (*c*, 1.0) (Found: C, 81.9; H, 11.3. $C_{31}H_{52}O_2$ requires C, 81.6; H, 11.4%). Further elution of the column with acetone-benzene gave intractable products.

28-Noreburic-8-en-21-ol (XXII; R = CH₂OH).—A solution of methyl 28-noreburic-8-en-21-oate (0.9 g.) in ether (150 ml.) containing lithium aluminium hydride (1 g.) was heated under reflux for 6 hr. Next day the excess of lithium aluminium hydride was destroyed with ethereal ethyl acetate, the mixture was poured into 2*N*-sulphuric acid, and the product was isolated with ether. Crystallised from methanol, this gave 28-noreburic-8-en-21-ol in needles (0.7 g.), m. p. 104—105.5°, $[\alpha]_D + 58^\circ$ (*c*, 1.6) (Found: C, 84.2; H, 12.1. $C_{30}H_{52}O$ requires C, 84.1; H, 12.1%).

28-Noreburic-8-en-21-al (XXII; R = CHO).—A mixture of 28-noreburic-8-en-21-ol (0.65 g.), benzoquinone (1.6 g.), aluminium *tert.*-butoxide (1.1 g.), and benzene (50 ml.) was heated under reflux for 14 hr. with the addition of more aluminium *tert.*-butoxide (0.5 g.) after 3 hr. This was poured into 2*N*-sulphuric acid (15 ml.), the excess of benzoquinone and *tert.*-butyl alcohol was removed with steam, the residue was extracted repeatedly with ether, and the combined extracts were washed with 0.5*N*-sodium hydroxide (50 ml. × 4) and then water (50 ml. × 4) and then dried. Crystallised from alcohol, the residue gave 28-noreburic-8-en-21-al (0.5 g.) in

plates, m. p. 126—128°, $[\alpha]_D +65^\circ$ (c , 1.1; l — 0.5 dm.) (Found: C, 84.3; H, 11.5. $C_{30}H_{50}O$ requires C, 84.5; H, 11.7%).

Lanost-8-ene (28-Noreburic-8-ene) (XXII; R = Me).—The hydrazone of the aldehyde (XXII; R = CHO) (0.2 g.) was reduced by the method used for 24-keto-28-noreburic-8-en-21-oate, and the product (0.18 g.) was chromatographed on aluminium oxide (17×1.2 cm.) from light petroleum (20 ml.). Elution with light petroleum (75 ml.) gave 28-noreburic-8-ene, m. p. 73—74°, $[\alpha]_D +67^\circ$ (c , 2.6; l = 0.5 dm.) (Found: C, 87.1; H, 12.7. Calc. for $C_{30}H_{52}$: C, 87.4; H, 12.6%). Thus obtained, 28-noreburic-8-ene was identical with a specimen of lanost-8-ene, m. p. 73—74°, $[\alpha]_D 66^\circ$ (c , 1.08; l = 0.5 dm.), prepared by the method of McGhie *et al.* (*loc. cit.*) from lanost-8-en-3-one (lanostenone) which was derived from lanost-8-en-3-ol (dihydrolanosterol) by the following procedure (cf. Ruzicka, Denss, and Jeger, *loc. cit.*). A mixture of lanost-8-en-3-ol (2 g.), cyclohexanone (5 ml.), aluminium *tert.*-butoxide (4 g.), and dioxan (30 ml.) was heated under reflux for 7 hr., cooled, and poured into 2N-sulphuric acid (30 ml.). After the removal of unchanged cyclohexanone and dioxan with steam the product (1.5 g.) was isolated with ether and chromatographed from light petroleum (100 ml.) on aluminium oxide (15×2 cm.). The fractions obtained by eluting the column successively with benzene-light petroleum (1 : 10; 100 ml.), (1 : 5; 50 ml.), and (4 : 5; 50 ml.) were combined and crystallised from acetone-methanol, giving lanost-8-en-3-one in plates (0.9 g.), m. p. 119—120°, $[\alpha]_D +68^\circ$ (c , 1.16) (Found: C, 84.9; H, 11.7. Calc. for $C_{30}H_{50}O$: C, 84.5; H, 11.7%).

Reduction of 3-Ketoeburico-8 : 24(28)-dien-21-oic Acid.—Sodium (8 g.) was added portionwise to a boiling solution of this acid (eburicodienonic acid, Part XVI) (0.35 g.) in alcohol (100 ml.) during 4 hr. with the addition of sufficient alcohol (in all 25 ml.) to maintain a clear solution. The mixture was poured into an excess of 2N-hydrochloric acid, the alcohol removed with steam, and the product collected, dried, and chromatographed from benzene (100 ml.) on neutralised aluminium oxide (26×1.5 cm.). The column was eluted with (a) benzene (800 ml.) and then acetone-benzene (b) (1 : 19; 250 ml.), (c) (1 : 9; 250 ml.), (d) (1 : 4; 1050 ml.), and (e) (1 : 1; 500 ml.). On evaporation fractions (a) and (b) gave a yellow gum (0.03 g.), and (c), (d), and (e) eburicic acid (0.01 g.), (0.2 g.), and (0.05 g.) which on crystallisation had m. p. 287—289°, identical with an authentic specimen (Found: C, 78.9; H, 10.5. Calc. for $C_{31}H_{50}O_3$: C, 79.1; H, 10.7%). Prepared by pyridine-acetic anhydride, the acetyl derivative had m. p. and mixed m. p. 252—254° (Found: C, 77.0; H, 9.9. Calc. for $C_{33}H_{52}O_4$: C, 77.3; H, 10.2%).

The authors thank J. G. Reynolds and R. A. G. Carrington of the Spectroscopic Section, Thornton Research Centre, Shell Petroleum Co. Ltd., for the measurement of infra-red spectra on solid films of lanost-8-ene derived from eburicic acid and lanosterol. One of them (R. S. W.) is indebted to the Department of Scientific and Industrial Research for a Maintenance Grant.