491. *The Chemistry of Fungi. Part XVIII.* The Cyclic System of Eburicoic Acid.*

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From nuclear transformations effected with eburicoic acid it is concluded that this compound contains a cyclic system comprising rings A, B, and C of the lanosterol molecule. The position of the hydroxyl group in ring A has been confirmed by cleavage of the system to a dibasic acid subsequently converted on pyrolysis into a five-membered cyclic ketone. Further, the position of the inert double bond relative to the hydroxyl group in eburicoic acid has been indicated by the conversion of methyl 3-hydroxy-7 : 11-diketoeburicanoate (IX; R = H) into methyl 7 : 11-diketo*iso*eburic-5-enoate (XI) with phosphorus pentachloride according to standard procedure.

Methyl 3-acetoxyeburic-8-enoate is converted into methyl 3-acetoxyeburic-7-enoate (XIII).

Oxidation of methyl 3-acetoxy-7 : 11-diketoeburic-8-enoate gives methyl 3-acetoxy-7 : 11 : 12-triketoeburico-5 : 8-dienoate (XVII). In methyl 3-acetoxy-7 : 11-diketoeburicanoate (IX; R = Ac) only the keto-group in the 7-position is reactive.

As in lanosterol the presence of angular methyl groups at $C_{(10)}$, $C_{(13)}$, and $C_{(14)}$ in eburicoic acid is deduced.

From the behaviour of methyl 3-hydroxyeburic-8-enoate † (dihydroeburicoate) (I) on treatment with phosphorus pentachloride it was suggested in Part XVI (*J.*, 1951, 2346) that the secondary hydroxyl group of eburicoic acid is in a six-membered terminal ring in the α-position to the carbon atom carrying the *gem*-dimethyl group. An examination of the oxidative scission of this ring according to the well-defined standard procedure has now provided evidence in confirmation of this view. Thus, condensation of methyl 3-ketoeburic-8-enoate (II; R = Me) with ethyl formate by sodium ethoxide gave a hydroxymethylene derivative (III; R = Me) which on oxidation with alkaline hydrogen peroxide furnished the monomethyl ester (VI; R = Me, R′ = R″ = H) of a tribasic acid. On pyrolysis this ester gave a keto-ester, methyl A-nor-2-ketoeburic-8-enoate (VII; R = Me, R′ = H), which has an infra-red absorption spectrum with a shoulder at 1733 $cm.^{-1}$ characteristic of a five-membered homocyclic ketone; the expected peak is partly masked by the absorption band due to the carbomethoxy-group at 1721 $cm.^{-1}$. Prepared by the pyrolysis of the tribasic acid (VI; R = R′ = R″ = H) the corresponding A-nor-2-ketoeburic-8-enoic acid (VII; R = R′ = H), however, gave an infra-red spectrum with bands at 1705 and 1733 $cm.^{-1}$ due respectively to carboxyl and carbonyl groups where the absorption characteristic of the five-membered cyclic ketone appears as a fully resolved peak.

In the absence of free mineral acid during the isolation process, the product from the condensation of the ethyl formate and the ester (II; R = Me) had m. p. 121—123° ($\lambda_{max.}$ 290 mμ, log ε 3·90) and with alcoholic ferric chloride gave instantaneously an intense ferric reaction. On treatment with a trace of hydrochloric acid this compound was converted into an isomeride, m. p. 153° ($\lambda_{max.}$ 275 mμ, log ε 4·02), which slowly developed a colour with alcoholic ferric chloride and, on treatment with alkali, regenerated the compound, m. p. 121—123°. On oxidation both compounds gave the ester (VI; R = Me, R′ = R″ = H). Clearly the product, represented as a formyl derivative by (IV), may also exist in two enolic forms (III) and (V). The structure and relations of the isomerides are being investigated but it may be noted that the intensity of the ultra-violet absorption spectra exhibited indicates the presence of enolic chromophores in both isomerides.

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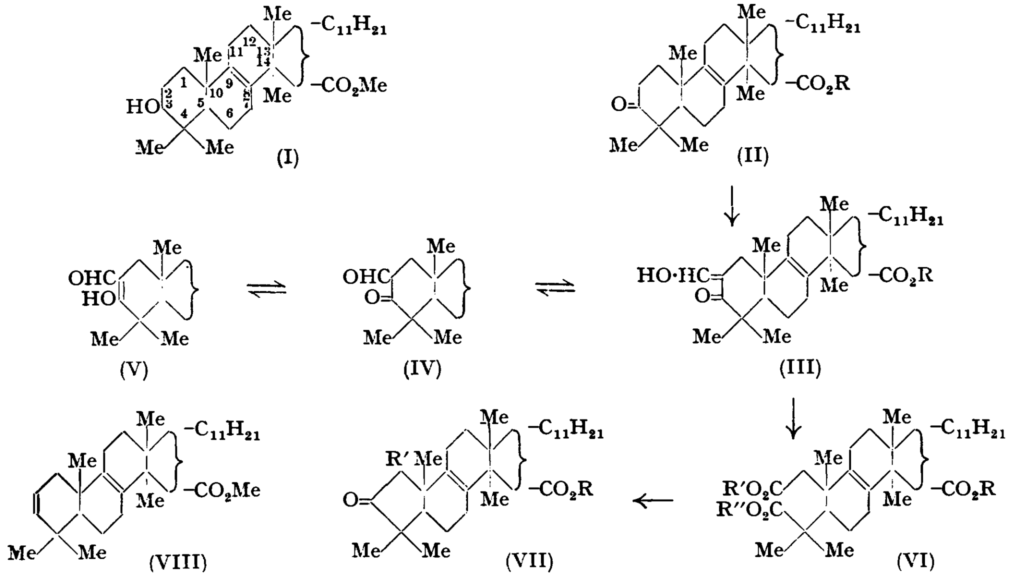
* Part XVII, *J.*, 1953, 1830.

† The relation of the cyclic system in eburicoic acid $C_{31}H_{50}O_3$ to that of lanosterol $C_{30}H_{50}O$ deduced in Part XVII * is clarified by the work described in the present communication. We are, therefore, adopting standard steroid numbering (see formula I) for rings A, B, and C. Where possible systematic nomenclature will now be used in which eburicanoic acid is the parent saturated system.

In keeping with the partial structure (VI; R = R′ = R″ = H) proposed for the tribasic acid formed by scission of (III; R = H) the trimethyl ester (VI; R = R′ = R″ = Me) of this acid is readily hydrolysed with methanolic potassium hydroxide to give a dimethyl ester which, in all probability, has the formula (VI; R = R″ = Me, R′ = H). The primary carbomethoxy-group arising from ring A would be preferentially attacked and under the conditions employed the carbomethoxy-group originally present is not hydrolysed (cf. Part XVI, *loc. cit.*) whilst the tertiary carbomethoxy-group from ring A would be expected to behave similarly. Along with the ester (VI; R = R″ = Me, R′ = H) there is formed methyl A-nor-2-ketoeburic-8-enoate which clearly arises from (VI; R = R′ = R″ = Me) by a Dieckmann condensation with subsequent hydrolysis and decarboxylation of the β-keto-ester (VII; R = Me, R′ = CO_2Me) initially formed.

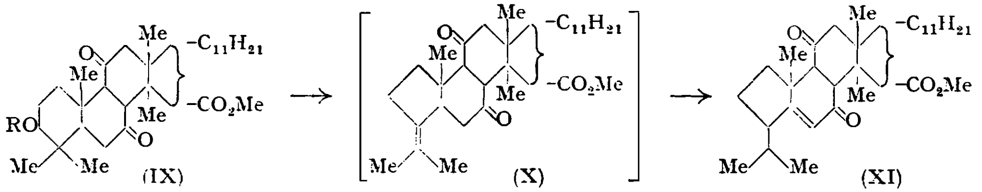


In contrast with the action of phosphorus pentachloride it has been found that on treatment with phosphoryl chloride methyl 3-hydroxyeburic-8-enoate (I) (dihydroeburicoate; Part XVI, *loc. cit.*) is dehydrated to a diene without the simultaneous transformation of ring A. Further, since this compound has an infra-red spectrum with a peak at 733 cm.$^{-1}$ characteristic of the system CHR:CHR (*cis*-form) it appears to be methyl eburico-2 : 8-dienoate (VIII) in agreement with its conversion into methyl eburic-8-enoate (methyl eburicenate; Part XVI) on hydrogenation.

In view of the similarities between the eburicoic acid and the lanosterol–agnosterol series (cf. also Part XVII) the following series of nuclear rearrangements and oxidations were undertaken. The results correspond closely to those observed with the lanosterol nucleus, and indicate clearly that rings A, B, and C of eburicoic acid and of lanosterol are structurally identical (cf. Dorée, McGhie, and Kurzer, *J.*, 1949, 570; Voser, Montavon, Günthard, Jeger, and Ruzicka, *Helv. Chim. Acta*, 1950, **33**, 1893; Cavalla, McGhie, and Pradhan, *J.*, 1951, 3142; Barton, Fawcett, and Thomas, *ibid.*, p. 3147).

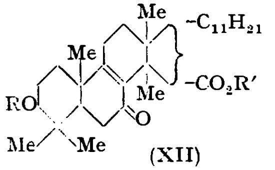
On reduction with zinc and acetic acid methyl 3-hydroxy-7 : 11-diketoeburic-8-enoate (dihydrodiketoeburicoate; Part XVII) gave rise to a saturated diketone, methyl 3-hydroxy-7 : 11-diketoeburicanoate (IX; R = H), which forms only a mono-2 : 4-dinitrophenylhydrazone ($\lambda_{max.}$ 361 mμ, log ε 4·39) indicating that one of the carbonyl groups is sterically hindered, as is the 11-keto-group in the sterol and lanosterol series. On dehydration with phosphorus pentachloride the diketone (IX; R = H) furnished methyl 7 : 11-diketo-*iso*eburic-5-enoate (XI). This conversion presumably arises by retropinacolic arrangement of (IX; R = H) into an intermediate of type (X) which, under acidic conditions is immediately transformed into (XI), where the double bond initially introduced, *i.e.*, in (X), becomes conjugated with a carbonyl group.

Methyl 7 : 11-diketo*iso*eburic-5-enoate gives only a mono-2 : 4-dinitrophenylhydrazone and from a consideration of the ultra-violet absorption spectrum of this derivative ($\lambda_{max.}$ 387 mμ, log ε 4·42) it is clear that the double bond is in conjugation with the more active carbonyl group which, therefore, is at $C_{(7)}$ in ring B. Hence, by analogy with the corresponding lanosterol derivatives (Barton *et al.*, Cavalla *et al.*, *locc. cit.*), the relation between the hydroxyl group in ring A and the carbonyl group in ring B of (IX) is established and, further, the $C_{(5)}$- and the $C_{(6)}$-atom each carry at least one hydrogen atom.

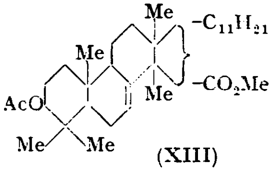


(IX) (X) (XI)

On mild oxidation with chromic anhydride 3-acetoxyeburic-8-enoic (*O*-acetyldihydroeburicoic; Part XVI) acid gave a mixture (cf. oxidation of *O*-acetyleburicoic acid; Part XVII) from which the main product, 3-acetoxy-7-ketoeburic-8-enoic acid (XII; R = Ac, R′ = H) was isolated by chromatography. The methyl ester (XII; R = Ac, R′ = Me) of this acid readily furnished a 2 : 4-dinitrophenylhydrazone ($\lambda_{max.}$ 390 mμ, log ε 4·47), thus showing that, as expected, it is the more reactive 7-carbonyl group in ring B which is first introduced by oxidation. Reduction of methyl 3-hydroxy-7-ketoeburic-8-enoate (XII; R = H, R′ = Me) by the Wolff–Kishner method followed by successive methylation and acetylation yielded an isomeride of methyl 3-acetoxyeburic-8-enoate in which the double bond is triply substituted (infra-red band at 823 cm.$^{-1}$). Since it has been shown (Lardelli and Jeger, *Helv. Chim. Acta*, 1949, **32**, 1817; Fischer, Lardelli, and Jeger, *ibid.*, 1950, **33**, 1335; 1951, **34**, 1577; Barton, Holness, and Klyne, *J.*, 1949, 2456) that the reduction of $\alpha\beta$-unsaturated ketones by this method is accompanied by the migration of the double bond to the carbon atom originally present in a carbonyl group, clearly this isomeride is methyl 3-acetoxyeburic-7-enoate (XIII).



(XII)



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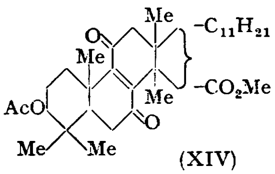

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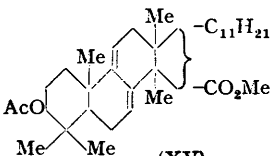
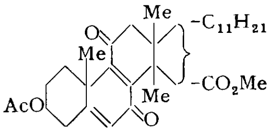


(XV)

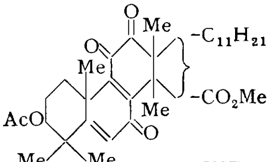
The compound (XIII) is also formed by the action of hydrogen chloride in acetic acid on methyl 3-acetoxyeburic-8-enoate. With a hydrogen atom at $C_{(10)}$ or $C_{(14)}$ it would be expected that under these conditions the double bond would migrate to the more stable $\Delta^{8(14)}$ or Δ^{9}-position and, therefore, the isolation of (XIII) in the absence of other reaction products indicates that $C_{(10)}$ and $C_{(14)}$ are fully substituted and it is suggested that each carries a methyl group. Methyl 3-acetoxyeburic-7-enoate could not be hydrogenated with hydrogen at atmospheric pressure and on oxidation with chromic anhydride gave methyl 3-acetoxy-7 : 11-diketoeburic-8-enoate (XIV) (*O*-acetyldihydrodiketoeburicoate, Part XVII), whilst with selenium dioxide it formed the heteroannular diene methyl 3-acetoxyeburico-7 : 9(11)-dienoate (XV) (*O*-acetyldehydrodihydroeburicoate, Part XVII).

On oxidation with selenium dioxide in boiling acetic anhydride methyl 3-acetoxy-7 : 11-diketoeburic-8-enoate (XIV) furnished a diketo-diene ($\lambda_{max.}$ 274 mμ, log ε 4·00) along with small amounts of a triketo-diene ($\lambda_{max.}$ 286 mμ, log ε 4·02) which, however, was obtained in better yield when the acetic anhydride was replaced by nitrobenzene. From a comparison of the ultra-violet absorption spectra of these oxidation products with the corresponding dihydrolanosterol derivatives the diketo-diene appears to be methyl 3-acetoxy-7 : 11-diketoeburico-5 : 8-dienoate (XVI), and the triketo-diene to be methyl 3-acetoxy-7 : 11 : 12-triketoeburico-5 : 8-dienoate (XVII) (Voser *et al.*, and Dorée *et al.*, *locc. cit.*). As with the triketone from lanosterol the failure to extend the conjugated system of (XVII) by further oxidation of this compound may be regarded as indicating that in addition to the *C*-methyl groups at $C_{(10)}$ and $C_{(14)}$ there is also a *C*-methyl group at $C_{(13)}$.

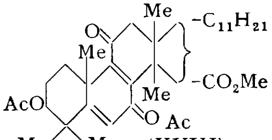
Attempts to oxidise (XIV) at the 8 : 9-double bond were unsuccessful. On reduction by the Meerwein–Ponndorf method this compound gave a product which on acetylation gave a diacetate, failed to form a 2 : 4-dinitrophenylhydrazone, and had $\lambda_{max.}$ 253 and 347—349 mμ (log ε 4·02, 2·74), thus indicating that, as expected, only the 7-carbonyl group was reduced. The acetate of the product is, therefore, methyl 3 : 7-diacetoxy-11-ketoeburic-8-enoate (XVIII) which, like (XIV), could not be oxidised at the Δ^8-position.



(XVI)



(XVII)



(XVIII)

The degradation experiments described are explained satisfactorily on the basis of the partial formulæ employed. On this account, and because of the similarities in the molecular-rotation differences of derivatives of eburicoic acid and lanosterol, where only changes in rings A, B, and C (already described in Part XVII) are involved, it appears that the nuclear system of eburicoic acid is structurally and stereochemically closely related to that of lanosterol.

The conversion of eburicoic acid into eburic-8-en-3-ol (dihydroeburicol) has been achieved by Lahey and Strasser (*J.*, 1951, 873). An alternative route to this alcohol is now described. Prepared from the corresponding acid, 3-acetoxyeburic-8-enoyl chloride was reduced by Rosenmund's method to 3-acetoxyeburic-8-enal, the hydrazone of which was reduced with simultaneous deacetylation by the Wolff–Kishner method to give eburic-8-en-3-ol. When the hydrazone was replaced by the semicarbazone in the latter reaction the alcohol was accompanied by a small amount of an isomeric compound.

EXPERIMENTAL

Specific rotations were measured in chloroform with a 1-dm. tube except where otherwise stated. Ultra-violet absorption spectra were determined in alcohol with a Unicam Spectrophotometer. Infra-red absorption measurements were made with a Grubb Parsons single-beam spectrometer and a paste of the material in "Nujol." Except where stated the light petroleum used had b. p. 60—80°.

Methyl 2-*Hydroxymethylene*-3-*ketoeburic*-8-*enoate* (III; R = Me).—Esterification of 3-keto-eburic-8-enoic acid (eburicenonic acid, Part XVI) with ethereal diazomethane gave the *methyl* ester (II; R = Me) which formed slender needles, m. p. 123—124°, $[\alpha]_D^{20}$ +61° (*c*, 2·0), from methanol (Found: C, 79·7; H, 11·0. $C_{32}H_{52}O_3$ requires C, 79·3; H, 10·7%).

A solution of methyl 3-ketoeburic-8-enoate (0·2 g.) and ethyl formate (0·4 g.) in ether (3·2 ml.) was added slowly to alcoholic sodium ethoxide (0·15 g. of sodium and 3·2 ml. of alcohol), and the cloudy mixture heated under reflux (moisture absent) for ½ hr., kept for 16 hr., and poured on ice and hydrochloric acid. On isolation an ethereal solution of the product was washed repeatedly to remove acid, dried, and evaporated, leaving *methyl* 2-*hydroxymethylene*-3-*ketoeburic*-8-*enoate* which separated from methanol in clusters of needles (0·15 g.), m. p. 121—123°, $[\alpha]_D^{20}$ +110° (*c*, 1·49), giving an instantaneous deep purple ferric reaction in alcohol (Found: C, 77·6; H, 10·3. $C_{33}H_{52}O_4$ requires C, 77·3; H, 10·2%). When the ethereal solution of the crude product was not completely freed from traces of hydrochloric acid evaporation of the dried solution then gave a compound forming needles, m. p. 153°, $[\alpha]_D^{20}$ +103° (*c*, 1·43) from methanol, which did not give an immediate reaction with alcoholic ferric chloride; the deep purple colour developed during ½ hr. (Found : C, 77·1; H, 10·0%). A mixture of the two compounds melted at about 116°. When a solution of the compound, m. p. 121—123°, in ether containing a trace of hydrogen chloride was kept for 15 min. and then evaporated the residue consisted of the compound, m. p. 153°, which, in turn, on treatment with 10% alcoholic sodium hydroxide for 15 min. regenerated the compound, m. p. 121—123°, provided care was taken in the isolation procedure to remove, from the ethereal solution of the product, traces of hydrochloric acid employed in the neutralisation of the alkaline reaction mixture which had been diluted with ice-water.

Oxidation of Methyl 2-Hydroxymethylene-3-ketoeburic-8-enoate with Hydrogen Peroxide.—Hydrogen peroxide (0·6 ml. of 100-vol.) and 10% aqueous sodium hydroxide were added to methyl 2-hydroxymethylene-3-ketoeburic-8-enoate (0·2 g.), dissolved in alcohol (3 ml.), and the mixture was heated on the steam-bath for 20 min. More 10% aqueous sodium hydroxide (3·3 ml.) and hydrogen peroxide (1·2 ml. of 100-vol.) were then added and next day the reaction mixture was acidified with dilute sulphuric acid and extracted with ether. From the combined ethereal extracts the *monomethyl* ester (VI; R = Me, R′ = R″ = H) of a tricarboxylic acid was isolated with 2N-sodium carbonate and purified from light petroleum containing a trace of alcohol, forming slender needles, m. p. 215—216°, $[\alpha]_D^{20}$ +62° (*c*, 0·72) [Found : C, 72·5; H, 10·0%; equiv. (by potentiometric titration in 50% alcohol), 274. $C_{32}H_{52}O_6$ requires C, 72·2; H, 9·8%; equiv., 266]. With ethereal diazomethane this compound gave the *trimethyl* ester (VI; R = R′ = R″ = Me) which separated from methanol at 0° in feathery needles, m. p. 78°, $[\alpha]_D^{20}$ +19° (*c*, 1·02) (Found : C, 73·2; H, 10·2. $C_{34}H_{56}O_6$ requires C, 72·9; H, 10·0%).

Hydrolysis of the monomethyl ester (1 g.) with boiling diethylene glycol (30 ml.) containing 10% of potassium hydroxide for 3 hr. gave the *tricarboxylic acid* (VI; R = R′ = R″ = H) which was isolated with ether from the cooled hydrolysate after dilution with water (150 ml.) and acidification with 2N-hydrochloric acid. Crystallised from acetic acid, this acid formed small needles, m. p. 278—280° (decomp.), $[\alpha]_D^{20}$ +36° (*c*, 0·97) (Found : C, 72·0; H, 9·6%; equiv., 176. $C_{31}H_{50}O_6$ requires C, 71·8; H, 9·7%; equiv., 173).

Pyrolysis of the Tricarboxylic Acid (VI; R = R′ = R″ = H) *and of its Monomethyl Ester.*—The acid (0·2 g.) was kept at 280—300° in nitrogen for 1 hr. and the product distilled at 210°/0·1 mm., giving A-*nor-2-ketoeburic-8-enoic acid* (VII; R = R′ = H) which separated from methanol in needles (0·07 g.), m. p. 258°, $[\alpha]_D^{20}$ +98° (*c*, 2·50) (Found : C, 78·9; H, 10·6. $C_{30}H_{48}O_3$ requires C, 78·9; H, 10·5%).

Pyrolysis of the monomethyl ester (0·13 g.) under the same conditions gave a product which was dissolved in light petroleum (20 ml.) and the solution poured on a column of aluminium oxide. Elution with light petroleum (40 ml.) gave *methyl* A-*nor-2-ketoeburic-8-enoate* (VII; R = Me, R′ = H) which separated from methanol in needles (0·06 g.), m. p. 185°, $[\alpha]_D^{20}$ +112° (*c*, 1·1; 0·5-dm. micro-tube) (Found : C, 79·2; H, 10·7. $C_{31}H_{50}O_3$ requires C, 79·1; H, 10·6%). Prepared by the pyridine method, the *oxime* separated from benzene–light petroleum in slender needles, m. p. 180—184° (Found : N, 2·9. $C_{31}H_{51}O_3N$ requires N, 2·9%).

The ester (VII; R = Me, R′ = H) was also formed by boiling the trimethyl ester (VI; R = R′ = R″ = Me) with 0·5N-alcoholic potassium hydroxide for 48 hr., and isolated with ether from the reaction mixture after dilution with water (100 ml.). The compound formed needles, m. p. and mixed m. p. 180—183°, $[\alpha]_D^{20}$ +118° (*c*, 2·2; 0·5-dm. micro-tube) (Found : C, 79·4; H, 10·6%), and gave the oxime, m. p. 180—184°, undepressed on admixture with an authentic specimen. After the isolation of this ester the residual alkaline liquor was acidified with sulphuric acid, and the precipitated product isolated with ether. Crystallised from methanol, this gave the *dimethyl* ester (VI; R = R″ = Me, R′ = H) which formed aggregates of thick needles, m. p. 150—152°, $[\alpha]_D^{20}$ +29° (*c*, 1·2), from methanol (Found : C, 72·6; H, 10·0%; equiv., 575. $C_{33}H_{54}O_6$ requires C, 72·5; H, 9·9%; equiv., 546). With ethereal diazomethane this compound gave the trimethyl ester, m. p. and mixed m. p. 78°, of the tribasic acid $C_{31}H_{50}O_6$.

Methyl Eburico-2 : 8-dienoate (VIII).—Phosphoryl chloride (5 ml.) was added slowly to a well-stirred solution of methyl 3-hydroxyeburic-8-enoate (I) (dihydroeburicoate, Part XVI) (3·3 g.) in pyridine (33 ml.), and the warm mixture (80°) heated on the steam-bath for 1½ hr., boiled for 3 min., cooled, and poured on crushed ice and excess of hydrochloric acid. After isolation with ether a solution of the product in light petroleum was poured on a column of aluminium oxide. Extraction of the oxide with excess of light petroleum gave *methyl eburico-*2 : 8-*dienoate* which separated from methanol in colourless needles (1 g.), m. p. 154—155°, $[\alpha]_D^{20}$ +105° (*c*, 1·92) (Found : C, 82·3; H, 11·3. $C_{32}H_{52}O_2$ requires C, 82·1; H, 11·1%). On hydrogenation in acetic acid at 100°/70 atm. with a platinum catalyst this compound gave methyl eburic-8-enoate in needles, m. p. 102° (Found : C, 81·6; H, 11·3. Calc. for $C_{32}H_{54}O_2$: C, 81·7; H, 11·5%). Thus obtained methyl eburic-8-enoate was identical with a specimen prepared by the hydrogenation of methyl eburico-8 : 24(28)-dienoate (eburicodienate, Part XVI) with hydrogen at atmospheric pressure and a platinum or W5 Raney nickel catalyst. Owing to an error in transcription the m. p. of this dihydro-derivative (methyl eburicenate) was erroneously stated in Part XVI to be 118—119·5°.

Methyl 3-*Hydroxy*-7 : 11-*diketoeburicanoate* (IX; R = H).—This compound was prepared by the reduction of methyl 3-hydroxy-7 : 11-diketoeburic-8-enoate (dihydrodiketoeburicoate, Part XVII) (1 g.) with zinc and acetic acid by the method employed for methyl 3-acetoxy-7 : 11-diketoeburic-8-enoate (methyl *O*-acetyldihydrodiketoeburicoate, Part XVII) and on purification from methanol formed colourless needles (0·6 g.), which contained solvent of crystallisation and melted at 113—115°, solidified at about 150—153°, and then melted at 176—177°. A solution of the solvated product in much light petroleum was evaporated until saturated and on cooling then deposited *methyl* 3-*hydroxy*-7 : 11-*diketoeburicanoate* in needles, m. p. 181—182°, $[\alpha]_D^{21}$ +43° (*c*, 1·31), $\lambda_{max.}$ 295 mμ (log ε 1·80) (Found : C, 74·6; H, 9·9. $C_{32}H_{52}O_5$ requires C, 74·4; H, 10·1%). The 2 : 4-*dinitrophenylhydrazone* separated from alcohol in red needles, m. p. 258—259° (decomp.) (Found : N, 8·2. $C_{38}H_{56}O_8N_4$ requires N, 8·0%). Hydrolysis of the ester with hot alcoholic potassium hydroxide gave an inseparable mixture.

Methyl 7 : 11-*Diketo*iso*eburic*-5-*enoate* (XI).—A mixture of phosphorus pentachloride (2 g.), methyl 3-hydroxy-7 : 11-diketoeburicanoate (0·7 g.), and light petroleum (b. p. 40—60°) (350 ml.) was agitated for 1 hr., filtered, washed with 2N-sodium hydroxide and water, dried, and concentrated to 100 ml. This solution was poured on aluminium oxide (24 × 1·8 cm.), and the column eluted with benzene–light petroleum (1 : 4; 700 ml.), giving *methyl* 7 : 11-*diketo*iso*buric*-5-*enoate* as a colourless resin (0·42 g.) which crystallised from methanol in colourless needles, m. p. 162—163°, $[\alpha]_D^{20}$ +18° (*c*, 2·91; 0·5-dm. micro-tube), $\lambda_{max.}$ 242, 295—296 mμ (log ε 3·87, 2·61) (Found : C, 76·3; H, 10·5. $C_{32}H_{54}O_4$ requires C, 76·4; H, 10·8%). The 2 : 4-*dinitrophenylhydrazone* formed red needles, m. p. 172—175°, from alcohol (Found : N, 7·8. $C_{38}H_{58}O_7N_4$ requires N, 8·2%). Further elution of the column with benzene–light petroleum (1 : 2) and acetone–benzene (1 : 19) gave yellow resins which did not crystallise from solvents.

3-*Acetoxy*-7-*ketoeburic*-8-*enoic Acid* (O-*Acetyldihydroketoeburicoic Acid*) (XII; R = Ac, R′ = H).—3-Acetoxyeburic-8-enoic acid (*O*-acetyldihydroeburicoic acid, Part XVI) (8·5 g.), dissolved in acetic acid (500 ml.), was oxidised with a solution of chromic anhydride (2·8 g.) in acetic acid (100 ml.) and water (20 ml.) at room temperature for 4 days and the product isolated by the method employed for *O*-acetylketoeburicoic acid (Part XVII). From the mixture of ketonic acids 3-*acetoxy*-7-*ketoeburic*-8-*enoic acid* was isolated by chromatography on neutralised aluminium oxide and purified from dilute alcohol, forming colourless needles (1·1 g.), m. p. 238—240°, $[\alpha]_D^{20}$ +12° (*c*, 2·65), $\lambda_{max.}$ 251 mμ (log ε 3·98) with an inflexion at about 315 mμ (log ε 1·85) (Found : C, 75·2; H, 10·1. $C_{33}H_{52}O_5$ requires C, 75·0; H, 9·9%). The *methyl* ester (XII; R = Ac, R′ = Me) formed needles, m. p. 164—165°, $[\alpha]_D^{23}$ +15° (*c*, 0·91), from methanol (Found : C, 75·6; H, 10·1. $C_{34}H_{54}O_5$ requires C, 75·2; H, 10·0%). The 2 : 4-*dinitrophenylhydrazone* of this keto-ester separated from alcohol in bright red needles, m. p. 240—243° (decomp.) (Found : N, 8·1. $C_{40}H_{58}O_8N_4$ requires N, 7·8%).

Deacetylation of 3-acetoxy-7-ketoeburic-8-enoic acid with boiling 10% alcoholic potassium hydroxide gave 3-*hydroxy*-7-*ketoeburic*-8-*enoic acid* (XII; R = R′ = H) which crystallised from aqueous alcohol in feathery needles, m. p. 273—274°, $[\alpha]_D^{21}$ +14° (*c*, 1·76), $\lambda_{max.}$ 251 mμ (log ε 3·93) with an inflexion at about 315 mμ (log ε 2·16) (Found, in a specimen sublimed in a high vacuum : C, 76·2; H, 10·2. $C_{31}H_{50}O_4$ requires C, 76·5; H, 10·4%).

Methyl 3-*Acetoxyeburic*-7-*enoate* (XIII).—(*a*) A mixture of 3-hydroxy-7-ketoeburic-8-enoic acid (0·5 g.), 90% hydrazine hydrate (0·5 ml.), and diethylene glycol (10 ml.) was boiled for ½ hr., treated with sodium (0·5 g.) and diethylene glycol (5 ml.), then boiled for 5 hr., cooled, and poured into 3N-hydrochloric acid (30 ml.). Next day the solid was isolated with ether and successively esterified with ethereal diazomethane and acetylated with acetic anhydride–pyridine. A solution of the resulting product in light petroleum (30 ml.) was poured on a column of aluminium oxide (20 × 2 cm.) which was washed with light petroleum (500 ml.)

followed by benzene–light petroleum [3 portions each of mixtures (300 ml.) 1 : 99; 1 : 9; and 2 : 3]. *Methyl* 3-*acetoxyeburic*-7-*enoate* was then eluted from the column with benzene (500 ml.) and on purification from methanol obtained in long needles (0·26 g.), m. p. 172—174°, $[\alpha]_D^{20}$ +30° (*c*, 3·12; 0·5-dm. micro-tube) (Found: C, 77·0; H, 10·4. $C_{34}H_{56}O_4$ requires C, 77·2; H, 10·7%).

(*b*) A solution of methyl 3-acetoxyeburic-8-enoate (methyl *O*-acetyldihydroeburicoate, Part XVI) (2 g.) in acetic acid (100 ml.) at 10° was saturated with hydrogen chloride and poured on ice-water (300 ml.). After crystallisation from methanol the product, long needles (1·79 g.), m. p. 164—168°, was dissolved in light petroleum (75 ml.) and adsorbed on a column of aluminium oxide (24 × 2 cm.). On being washed with successive portions of solvent the chromatogram gave the following fractions which were crystallised from methanol: (1) with 300 ml. of light petroleum unchanged methyl 3-acetoxyeburic-8-enoate (0·29 g.), m. p. 157°; (2) 100 ml. of light petroleum, solid (0·17 g.), m. p. 163°; (3) 50 ml., solid (0·06 g.), m. p. 165°; (4)—(11) 1500 ml. of light petroleum, solid (0·5 g.), m. p. 167°; (12) 100 ml. of acetone–light petroleum (1 : 99), solid (0·02 g.), m. p. 167°; (13) 100 ml. of acetone–light petroleum (1 : 19), solid (0·71 g.), m. p. 166—168°; (14) 100 ml. of same mixture, oil (0·02 g.). The fractions (4)—(13) were combined and repeatedly crystallised from methanol, giving methyl 3-acetoxyeburic-7-enoate in long, colourless needles (1·02 g.) which gave a yellow colour with tetranitromethane in chloroform and had m. p. and mixed m. p. 172—174°, $[\alpha]_D^{20}$ +34° (*c*, 1·60) (Found: C, 77·3; H, 10·6%).

This compound could not be hydrogenated in acetic acid with hydrogen at atmospheric pressure and a platinum catalyst and, after treatment with hydrogen chloride in acetic acid, was recovered unchanged.

Oxidation of Methyl 3-*Acetoxyeburic*-7-*enoate* (XIII).—(*a*) A solution of this ester (0·5 g.), chromic anhydride (0·4 g.), acetic acid (15 ml.), and water (1 ml.) was kept at 80° for 1 hr., treated with a little methanol to destroy unchanged anhydride, and diluted with water (200 ml.). A solution of the yellow solid in benzene–light petroleum (1 : 1) (20 ml.) was poured on aluminium oxide (12 × 1·8 cm.). Eluted with acetone–benzene (1 : 19) (250 ml.), the resulting methyl 3-acetoxy-7 : 11-diketoeburic-8-enoate (XIV) separated from light petroleum or methanol in squat yellow prisms (0·39 g.), m. p. 171—172°, $[\alpha]_D^{20}$ +75° (*c*, 2·39; 0·5-dm. micro-tube), $\lambda_{max.}$ 270 mμ (log ε 3·92), identical with an authentic specimen.

(*b*) A mixture of methyl 3-acetoxyeburic-7-enoate (1 g.), selenium dioxide (1·5 g.), acetic acid (20 ml.), and water (0·25 ml.) was heated under reflux for 3 hr., cooled, filtered, and diluted with water (100 ml.). A solution of the solid in light petroleum (50 ml.) was poured on aluminium oxide (60 × 2 cm.), and the column then eluted with much light petroleum, giving methyl 3-acetoxyeburico-7 : 9(11)-dienoate (methyl *O*-acetyldehydrodihydroeburicoate, Part XVII) (XV) which crystallised from methanol in long, colourless needles (0·68 g.), m. p. 165—166°, $[\alpha]_D^{20}$ +66° (*c*, 1·77), $\lambda_{max.}$ 234, 243, 252 mμ (log ε 4·22, 4·28, and 4·11 respectively), identical with an authentic specimen.

Oxidation of Methyl 3-*Acetoxy*-7 : 11-*diketoeburic*-8-*enoate* (XIV) *with Selenium Dioxide*.—(*a*) A mixture of this ester (0·5 g.), selenium dioxide (1 g.), and nitrobenzene (5 ml.) was heated under reflux for 2 hr. and filtered, and the selenium and selenium dioxide were washed with ether. The combined filtrate and washings were extracted with 2N-sodium hydroxide (50 ml. × 5), then with 2N-hydrochloric acid, and finally with water. After the removal of the ether and nitrobenzene with steam the residual brown gum was dissolved in benzene–light petroleum (1 : 4) and the solution poured on aluminium oxide (16 × 1·3 cm.). Elution of the column in turn with benzene–light petroleum (1 : 4; 700 ml., and 800 ml.), and acetone–benzene (1 : 10; 500 ml.) gave three fractions which were combined and crystallised from alcohol, giving *methyl* 3-*acetoxy*-7 : 11 : 12-*triketoeburico*-5 : 8-*dienoate* (XVII) in orange-yellow needles (0·22 g.), m. p. 165—166°, $[\alpha]_D^{20}$ −79° (*c*, 0·45), $\lambda_{max.}$ 216, 286 mμ (log ε 4·04, 4·02) (Found: C, 71·6; H, 8·6. $C_{34}H_{48}O_7$ requires C, 71·8; H, 8·5%).

When this oxidation was repeated with more methyl 3-acetoxy-7 : 11-diketoeburic-8-enoate (4·5 g.) the fraction (2·97 g.) eluted from the column with benzene–light petroleum (1 : 4) and subsequently with benzene was the expected triketonic ester but a later fraction eluted with acetone–benzene (1 : 10) consisted of a compound believed to be *methyl* 3-*acetoxy*-6 : 7 : 11 : 12-*tetraketoeburic*-8-*enoate* which separated from aqueous alcohol in orange needles (0·12 g.), m. p. 194·5—195·5°, $[\alpha]_D^{23}$ −45° (*c*, 1·03), $\lambda_{max.}$ 294, 412—414 mμ (log ε 3·80, 2·51) (Found: C, 69·5; H, 8·7. $C_{34}H_{48}O_8$ requires C, 69·8; H, 8·3%). In a subsequent experiment with 21 g. of starting material this compound was not obtained; the only crystalline product was the triketonic ester (yield, 35%).

(*b*) A solution of methyl 3-acetoxy-7 : 11-diketoeburic-8-enoate (0·5 g.) in acetic anhydride (5 ml.), containing selenium dioxide (0·5 g.) was boiled for 3 hr., filtered (the solid washed with acetic acid), and poured into water. An ethereal solution of the resulting yellow product was extracted successively with 2N-sodium hydroxide (30 ml. × 5), 2N-hydrochloric acid, and water, dried, and evaporated. By chromatography from benzene–light petroleum (1 : 1; 50 ml.) on aluminium oxide (25 × 1·3 cm.) the residue was resolved into 2 fractions. Eluted with benzene–light petroleum (1 : 1, 50 ml.), fraction (1), a yellow gum mixed with needles (0·29 g.), consisted of *methyl* 3-*acetoxy*-7 : 11-*diketoeburico*-5 : 8-*dienoate* (XVI) which separated from methanol and then aqueous methanol in yellow needles (0·22 g.), m. p. 144—145°, $[\alpha]_D^{21}$ −1° (*c*, 1·12), $\lambda_{max.}$ 274 mμ (log ε 4·00), with an inflexion at about 370 mμ (log ε 2·18) (Found, in specimen sublimed in a high vacuum : C, 73·7; H, 8·9. $C_{34}H_{50}O_6$ requires C, 73·6; H, 9·1%).

Obtained by washing the column with more benzene–light petroleum (1 : 1; 200 ml.), fraction (2) was an orange gum (0·05 g.) which, on crystallisation from light petroleum and then alcohol, gave methyl 3-acetoxy-7 : 11 : 12-triketoeburico-5 : 8-dienoate in orange-yellow needles, m. p. 164—165°, identical with a specimen prepared by method (*a*).

Oxidation of methyl 3-acetoxy-7 : 11-diketoeburico-5 : 8-dienoate (0·08 g.) with selenium dioxide (0·08 g.) in boiling nitrobenzene (2 ml.) for 2 hr. gave a product from which only methyl 3-acetoxy-7 : 11 : 12-triketoeburico-5 : 8-dienoate was isolated by chromatography on aluminium oxide. Crystallised from alcohol, this formed orange-yellow needles (0·05 g.), m. p. 165—166°, $\lambda_{max.}$ 216, 286 mμ (log ε 4·05, 3·99), identical with an authentic specimen.

Reduction of Methyl 3-*Acetoxy*-7 : 11-*diketoeburic*-8-*enoate* (XIV).—On the addition of aluminium *iso*propoxide (30 g.) in *iso*propanol (75 ml.) the yellow solution of this ester (5 g.) in dioxan (50 ml.) became reddish-orange and was then heated on the steam-bath for 25 hr. with slow distillation of the solvent which was replaced by additions of *iso*propanol. Acetone (2 : 4-dinitrophenylhydrazone) was detected in the distillate during the first 8 hr.; the reaction was also followed by determinations of the ultra-violet spectrum of samples withdrawn at intervals, changing from $\lambda_{max.}$ 256—257 mμ after 9 hr. to $\lambda_{max.}$ 254 mμ ($E_{1\,cm.}^{1\%}$ cm. 141) after 17 hr. and $\lambda_{max.}$ 254 mμ ($E_{1\,cm.}^{1\%}$ cm. 143) after 25 hr. The mixture was then treated with an excess of 2N-hydrochloric acid, and the solid chromatographed on aluminium oxide from benzene (100 ml.). The combined fractions eluted with benzene (100 and 450 ml.), then acetone–benzene (1 : 10, 250 ml.; and 1 : 3, 100 ml.) which consisted of colourless gums (4·42 g.), $\lambda_{max.}$ 254—255 mμ, were dissolved in alcohol, and the solution was poured into water, giving a colourless granular precipitate, m. p. 185—190°, which did not crystallise. This was acetylated by the pyridine method and on crystallisation from alcohol and then light petroleum the product gave *methyl* 3 : 7-*diacetoxy*-11-*ketoeburic*-8-*enoate* (XVIII) in colourless needles (3·3 g.), m. p. 147—148°, $[\alpha]_D^{20}$ +102° (*c*, 1·05) (Found : C, 71·9; H, 9·4. $C_{36}H_{56}O_7$ requires C, 72·0; H, 9·4%). Under the usual conditions this compound did not react with 2 : 4-dinitrophenylhydrazine.

3-*Acetoxyeburic*-8-*enal*.—A mixture of 3-acetoxyeburic-8-enoic acid (*O*-acetyldihydroeburicoic acid, Part XVI) (5 g.), thionyl chloride (10 g.), and benzene (100 ml.) was heated under reflux for 3 hr. and evaporated, leaving 3-*acetoxyeburic*-8-*enoyl chloride* which separated from absolute acetone in colourless needles (3·5 g.), m. p. 175—177° (Found : C, 74·2; H, 9·7; Cl, 7·1. $C_{33}H_{53}O_3Cl$ requires C, 74·3; H, 10·0; Cl, 6·7%).

Hydrogen was led into a boiling solution of this chloride (2 g.) in *o*-xylene (200 ml.), containing 5% palladium–barium sulphate (2 g.), during 5 hr.; determination of the hydrogen chloride evolved with standard aqueous sodium hydroxide indicated that the reaction was complete. The mixture was evaporated in nitrogen under diminished pressure, the residue (1·8 g.) dissolved in light petroleum (100 ml.), and the solution poured on aluminium oxide (24 × 2 cm.). Elution with light petroleum (1·2 l.) gave 3-*acetoxyeburic*-8-*enal* which separated from alcohol in colourless leaflets (0·8 g.), m. p. 152—153° (decomp.), containing solvent of crystallisation. Sublimed in a high vacuum, the compound had m. p. 152—153°, $[\alpha]_D^{20}$ +70° (*c*, 3·0) (Found : C, 79·4; H, 10·7. $C_{33}H_{54}O_3$ requires C, 79·5; H, 10·9%). The *semicarbazone* formed needles, m. p. 263—265°, from alcohol (Found : N, 7·4. $C_{34}H_{57}O_3N_3$ requires N, 7·6%).

Eburic-8-*en*-3-*ol*.—(*a*) A mixture of 3-acetoxyeburic-8-enal (0·75 g.), potassium hydroxide (0·7 g.), 90% hydrazine hydrate (0·5 ml.), and diethylene glycol (5 ml.) was heated under reflux for 1½ hr., the excess of hydrazine hydrate distilled off, and the residue kept at 195° for 4 hr., cooled, diluted with water, and acidified. A solution of the dried precipitate (0·59 g.) in light petroleum was poured on a column of aluminium oxide (18 × 2 cm.) from which only traces of solid were obtained by washing successively with light petroleum, benzene, and acetone–benzene (1 : 19). Elution with acetone–benzene (1 : 9) then gave a crystalline solid (0·45 g.) which on recrystallisation from benzene–methanol (1 : 19) gave eburic-8-en-3-ol in needles, m. p. 166—

167°, $[\alpha]_D^{20}$ +54° (*c*, 3·2) (Found : C, 84·2; H, 12·3. Calc. for $C_{31}H_{54}O$: C, 84·1; H, 12·3%). The acetate separated from alcohol in plates, m. p. 130—131°, $[\alpha]_D^{20}$ +56° (*c*, 2·0) (Found : C, 81·9; H, 11·8. Calc. for $C_{33}H_{56}O_2$: C, 81·8; H, 11·6%).

(*b*) A mixture of 3-acetoxyeburic-8-enal semicarbazone (1·0 g.) and alcoholic sodium ethoxide (from 1·8 g. of sodium and 40 ml. of alcohol) was heated to 185° for 10 hr., cooled, diluted with water, and acidified with sulphuric acid. Crystallised from alcohol the precipitate gave eburic-8-en-3-ol in needles (0·32 g.), m. p. and mixed m. p. 166—167°, $[\alpha]_D^{20}$ +57° (*c*, 2·7) (Found : C, 84·2; H, 12·5%).

Concentration of the alcohol liquors from eburic-8-en-3-ol gave a small amount of a second product (50 mg.) which on repeated purification from alcohol had m. p. 165—166°, $[\alpha]_D^{20}$ +38° (*c*, 1·6) (Found: C, 84·5; H, 12·4%), and on admixture with eburic-8-en-3-ol had m. p. 155—157°

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