521. *The Chemistry of Fungi. Part XVI.* Eburicoic Acid.*

By R. M. Gascoigne, J. S. E. Holker, B. J. Ralph, and Alexander Robertson.

From the mycelium of the following fungi grown on a synthetical medium a triterpene acid, $C_{30}H_{48}O_3$, eburicoic acid, has been isolated—*Polyporus anthracophilus* Cooke, *P. eucalyptorum* Fr., *P. sulphureus* (Bull) Fr., *Fomes officinalis* Fr., and *Lentinus dactyloides* Clel. The acid from the last two mentioned sources was accompanied by a compound of the same type, containing a conjugate system of double bonds, whilst from mycelium of *P. anthracophilus* Cooke grown naturally on *Eucalyptus regnans* wood the acetyl derivative of eburicoic acid was isolated.

Eburicoic acid is a triterpenoid hydroxy-acid having a tertiary carboxyl group and two double bonds, of which one is easily hydrogenated and is present in a methylene group since on ozonolysis *O*-acetyleburicoic acid gives formaldehyde. In confirmation of this, scission of the diol formed from methyl *O*-acetyleburicoate gave rise to a compound believed to be a ketone along with formaldehyde. Eburicoic acid was converted into the hydrocarbon eburicene-I by a series of standard reactions. Similarly, it appears that the hydroxyl group is located in a terminal ring system and is in the α-position to the carbon atom carrying the *gem*-dimethyl group. It is concluded that eburicoic acid probably belongs to the tetracyclic series of triterpenes.

In the course of studies at present in progress in these laboratories on the metabolic products of a considerable number of the higher fungi, mainly of the class Basidiomycetes, grown on a synthetical medium, a sample of *Eucalyptus regnans* wood rotted by the fungus *Polyporus anthracophilus* Cooke was examined. From the mass of mycelial hyphæ encompassing the decayed wood the acetyl derivative of a monobasic acid, m. p. 292°, was isolated (*cf.* Gascoigne *et al.*, *Nature* 1950, **166**, 652; 1951, **167**, 570) which, from its properties and composition, appeared to belong to the triterpene class. When the fungus responsible for the rot was isolated and grown on a synthetical medium the mycelium contained only the parent acid and not the expected acetate. A preliminary investigation of the acid and some of its derivatives (*loc. cit.*) indicated that the compound had the empirical formula $C_{27}H_{44}O_3$, but, from a subsequent more detailed examination of the compound together with its derivatives and degradation products described in the present communication, it became clear that the acid had the formula $C_{30}H_{48}O_3$. From an examination of the literature it appeared that since the methyl esters and their acetates had similar melting points this acid might be identical with an acid, $C_{30}H_{48}O_3$, m. p. 283°, obtained by Kariyone and Kurono (*J. Pharm. Soc. Japan*, 1940, **60**, 110, 318) from the naturally grown fungus *Fomes officinalis* Fr., which these authors had named eburicoic acid. Accordingly the fungus *Fomes officinalis* Fr. was cultivated on the standard medium and from the mycelium an acid was isolated which differed from the product of *P. anthracophilus* Cooke in showing selective absorption in the ultra-violet with a peak at 243 mμ. and associated subsidiary peaks at 236 mμ. and 251 mμ. From the low intensity of the absorption, $E^{1\%}_{1\,\text{cm.}}$ 37·8, it appeared that the product was a mixture consisting mainly of eburicoic acid along with a small amount of an unsaturated analogue which was extremely difficult to remove. By chromatography of the acetylated mixture on neutralised aluminium oxide with subsequent deacetylation a small amount of an acid, m. p. 292°, was obtained which was identical in every way with the acid obtained from decayed *E. regnans* and from the mycelium of artificially cultivated *P. anthracophilus*. Accordingly, therefore, we propose to retain the name eburicoic acid for this compound but are at present unable to say whether the product described by the Japanese workers contained the unsaturated impurity. It is of

	Kariyone and Kurono's acid.		Acid from *P. anthracophilus* Cooke.	
	M. p.	$[\alpha]_D$.	M. p.	$[\alpha]_D$.
Acid	283°	—	292°	—
Methyl ester	141	+37·2°	140—141	+42·4°
Acetate	240	+80	256—257	+48·1
Methyl ester acetate	150	+56·9	154—155	+48·7

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interest to note that the naturally grown *Fomes officinalis* Fr. is a source of agaracinic acid but when grown on the synthetical medium the fungus does not form this product. As Kariyone and Kurono (*loc. cit.*) employed a hydrolytic process in the isolation of their product it is not clear whether the eburicoic acid contained in it was originally present in the free state or as the acetate. In this connection it may be noted that numerous earlier workers have examined natural *Fomes officinalis* Fr. and on a number of occasions less pure forms of the product obtained by Kuriyone and Kurono have been described (*cf.* Berzelius, " Lehrbuch der Chemie," 1838, **7**, 448; Thom and Vogelsang, *Annalen*, 1907, **357**, 145).

In the examination of the metabolic products of other Basidiomycetes grown on the standard medium, eburicoic acid has been isolated from *Polyporus eucalyptorum* Fr., *P. sulphureus* (Bull) Fr. (*cf.* Zellner and Zikmunda, *Monatsh.*, 1930, **56**, 200, who obtained a compound, m. p. 265°, from the naturally grown fungus, which may have been the impure acid), and *Lentinus dactyloides* Clel. The acid from the last-mentioned fungus also contained the unsaturated contaminant ($E^{1\%}_{1\,\text{cm.}}$ at 243 mμ. ranging from 99 to 125 in different batches) which was removed by chromatography. The value of the $\lambda_{\text{max.}}$ together with the structure of the ultra-violet absorption curve of the mixture clearly indicates that the contaminant contains a conjugate system of double bonds (*cf.* Booker *et al.*, *J.*, 1940, 1453, and Woodward, *J. Amer. Chem. Soc.*, 1942, **64**, 72), and work in progress indicates that this compound is closely related to eburicoic acid. The difficulties encountered in separating the natural mixture are reminiscent of the non-separable mixtures of lanosterol and agnosterol with their dihydro-compounds (Ruzicka *et al.*, *Helv. Chim Acta*, 1945, **28**, 759; 1946, **29**, 204).

Since eburicoic acid is not esterified by the application of the Fischer–Speier method, and the methyl ester formed with diazomethane is not readily hydrolysed, a property observed by Kariyone and Kurono (*loc. cit.*), it seems likely that the carboxyl group is tertiary. From the behaviour of methyl *O*-acetyleburicoate with perbenzoic acid it is clear that the parent acid contains two double bonds, of which one is easily hydrogenated, giving rise to dihydroeburicoic acid; the second double bond appears to resist hydrogenation. The reactive double bond is clearly present in a methylene group since on ozonolysis *O*-acetyleburicoic acid gave a comparatively good yield of formaldehyde whereas *O*-acetyldihydroeburicoic acid furnished only insignificant amounts. The main ozonolysis product of *O*-acetyleburicoic could not be crystallised but when methyl *O*-acetyleburicoate was oxidised with osmium tetroxide and the resulting diol, $C_{33}H_{54}O_6$, submitted to the action of lead tetra-acetate, formaldehyde was formed along with a crystalline compound believed to be the ketone, $C_{32}H_{50}O_5$, the absorption spectrum of which indicated that it was not an αβ-unsaturated ketone. With the presence of two double bonds in the molecule it seems that eburicoic acid belongs to the tetracyclic triterpene series, and a number of its properties are similar to those of well-known members of this group—lanosterol, agnosterol, elemadienolic acid, and euphol. From preliminary experiments on the dehydrogenation of eburicoic acid with selenium the main hydrocarbon formed appears to be a tri- or tetra-methylphenanthrene, a result typical of the tetracyclic group. It differs, however, from these compounds in yielding formaldehyde and not acetone on ozonolysis but in this respect it resembles basseol and the other triterpenoid acids isolated from fungi, *viz.* the polyporenic acids which also contain one inert and one reactive double bond (Cross *et al.*, *J.*, 1940, 632, 1491; Morgan, *Biochem. J.*, 1951, **48**, xliii). Eburicoic acid, however, does not cyclise under the conditions by which basseol is converted into β-amyrin.

In an attempt to relate eburicoic acid more closely to known triterpenes it was converted into the hydrocarbon eburicene-I.* By Oppenauer's method with *cyclo*hexanone in dioxan as the oxidising agent, eburicoic acid yielded a keto-acid, eburicodienonic acid, the absorption spectrum of which (inflexion at 282 mμ., log ε 1·5) indicates that the keto-group was not conjugated. By reduction according to the Wolff–Kishner method the hydrazone of this keto-acid gave eburicodienic acid and on hydrogenation the methyl ester of this compound furnished methyl eburicenate which with lithium aluminium hydride yielded the alcohol eburicenol. With *p*-benzoquinone as the oxidising agent the oxidation of eburicenol according to Oppenauer gave rise to the aldehyde eburicenal, the semicarbazone of which was reduced by the Wolff–Kishner method to eburicene-I. When, however, the semicarbazone was replaced by the hydrazone in this reaction eburicene-I was accompanied by a small amount of an isomeric hydrocarbon eburicene-II, neither of which appears to be identical with any of the known triterpenoid hydrocarbons.

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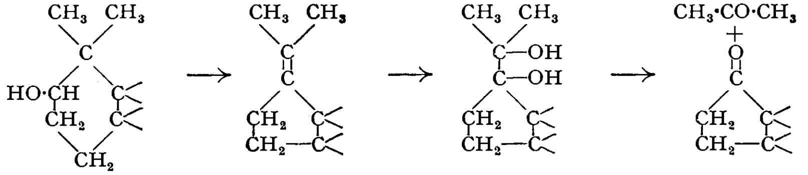
* The rational nomenclature is based on the name eburicane for the hypothetical fully saturated hydrocarbon $C_{30}H_{54}$. Thus eburicoic acid becomes eburicodienolic acid and its dihydro-derivative eburicenolic acid.

In the exploration of the nature of eburicoic acid the compound was submitted to the following series of reactions which have been used to elucidate the structure of ring A in several well-known triterpene compounds. Dehydration of methyl dihydroeburicoate (I) with phosphorus pentachloride gave methyl eburicodienate-II (II), and with osmium tetroxide this compound furnished a diol (III) which on oxidation with lead tetra-acetate gave a high yield of acetone along with a ketone (IV). Although this ketone did not crystallise, the well-defined semicarbazone and 2 : 4-dinitrophenylhydrazone served to establish clearly its formula as $C_{28}H_{44}O_3$. The absorption spectrum of the semicarbazone ($\lambda_{max.}$ 225 mμ.; log ε 4·2) indicated that the keto-group was not conjugated. In this connection it may be noted that the absorption spectrum of methyl eburicotrienate, formed by the dehydration of methyl eburicoate, indicated the absence of a conjugated system.

The formation of acetone from methyl eburicodienate-II in the foregoing manner suggests that the dehydration of methyl dihydroeburicoate proceeds in the same way as in a number of pentacyclic triterpenoids and also in the tetracyclic lanosterol (Ruzicka *et al.*, *Helv. Chim. Acta*, 1948, **31**, 818; Dorée *et al.*, *J.*, 1949, S167) where in the dehydration process a retropinacolic rearrangement is postulated resulting in the contraction of the ring originally containing the hydroxyl group, with the formation of an *iso*propylidene group :



(I.) (II.) (III.) (IV.)

This rearrangement requires that the hydroxyl group is located in a terminal ring in the α-position to the carbon atom carrying the *gem*-dimethyl group.

EXPERIMENTAL.

The melting points given are uncorrected and all analytical specimens were dried to constant weight in a high vacuum at elevated temperatures. Alcoholic solutions of the compounds were employed for the absorption-spectra measurements.

O-Acetyleburicoic Acid from Eucalyptus regnans.—A sample of decayed wood of *Eucalyptus regnans* which was kindly supplied to us by Mr. J. Somerville of the Australian Newsprint Mills, Boyer, consisted of a fine white powder containing fragments of wood and fungus mycelium. This was extracted with boiling alcohol and on cooling the concentrated extract gave *O*-acetyleburicoic acid in clusters of long silky needles which on recrystallisation had m. p. 256—257°, $[\alpha]^{25}_{D}$ +33·4° (*c*, 3·1 in pyridine) and was soluble in the usual organic solvents except light petroleum (Found : C, 77·0; H, 10·0. Calc. for $C_{32}H_{50}O_4$: C, 77·0; H, 10·1%). Deacetylation of *O*-acetyleburicoic acid with boiling 10% alcoholic potassium hydroxide yielded eburicoic acid which crystallised from alcohol in felted masses of slender needles, m. p. 292° (Found : C, 78·9; H, 10·5. Calc. for $C_{30}H_{48}O_3$: C, 78·9; H, 10·6%). Prepared with ethereal diazomethane, methyl *O*-acetyleburicoate formed silky needles, m. p. 154—155°, from methanol [Found : C, 77·3; H, 10·3; OMe, 5·7. Calc. for $C_{32}H_{49}O_3(OMe)$: C, 77·3; H, 10·2; OMe, 6·0%]. On being boiled with 10% methanolic potassium hydroxide (50 ml.) for 1 hour this compound (0·2 g.) gave methyl eburicoate which separated from methanol in slender needles, m. p. 140—141° [Found : C, 79·2; H, 10·6; OMe, 6·9. Calc. for $C_{30}H_{47}O_2(OMe)$: C, 79·1; H, 10·7; OMe, 6·6%].

Perbenzoic Acid Titration of Methyl Acetyleburicoate.—To the ester (99·5 mg.), dissolved in pure chloroform (20 ml.), at 0° a solution of perbenzoic acid in chloroform (5 ml.; 0·5N.) was added. The mixture was kept at 0° and samples withdrawn and treated with a mixture of potassium iodide (0·5 g.) and acetic acid (3 ml.) in water (20 ml.) which was then titrated with 0·1005N-sodium thiosulphate solution, starch indicator being used. A blank determination was carried out at the same time and under the same conditions. After 1 day 1·97 equivalents and after 2 days 2·18 equivalents of perbenzoic acid were consumed; the latter did not change after 7 days.

Eburicoic Acid from Fungi Cultivated on a Synthetical Medium.—The following fungi were grown on a modified Williams–Saunders medium (*Biochem. J.*, 1934, **28**, 1887) containing " Marmite " (0·1 g. per litre) with glucose (100 g. per litre) in place of sucrose and glycine (2 g. per litre) in place of asparagine. The identification of the cultures was checked for us by Professor J. Westerdijk of the Centraalbureau voor Schimmelcultures, Baarn, to whom our thanks are due.

The washed mycelium of each species was dried in a vacuum at 50°, milled to a fine powder, and extracted in a Soxhlet, first with light petroleum (b. p. 40—60°) to remove fat and then exhaustively with ether. The residue left on evaporation of the ethereal extract was purified by repeated crystallisation from alcohol until a product of constant melting point was obtained. The yields of eburicoic acid given are those of once crystallised material and are expressed as a percentage of the weight of dried mycelium.

(a) *From* Polyporus anthracophilus *Cooke*. The product (yield *ca.* 10%) was contaminated with a small amount of a low-melting non-acidic compound which could not be satisfactorily removed by recrystallisation from alcohol. Consequently the acid was converted into the sodium salt (see below) which after having been extracted several times with boiling benzene was decomposed, giving eburicoic acid which crystallised from alcohol in colourless slender needles, m. p. 292°, $[\alpha]_{\mathrm{D}}^{29}$ +35·9° (*c*, 1·8 in pyridine) (Found : C, 78·8; H, 10·4. Calc. for $C_{30}H_{48}O_3$: C, 78·9; H, 10·6%), moderately soluble in polar solvents and slightly soluble in non-polar solvents. In the Liebermann–Burchard reaction the acid gave a stable ruby-red coloration. The Salkowski and Rosenheim tests with the acid were negative. The *sodium* salt was prepared by treatment of the pure or partially purified acid with a hot mixture of equal volumes of 2N-aqueous sodium hydroxide and alcohol, followed by the addition of water. The cooled mixture deposited the salt in long glistening needles which were recrystallised from warm aqueous alcohol containing a trace of sodium hydroxide (Found : Na, 4·9, 4·7. $C_{30}H_{47}O_3Na$ requires Na, 4·8%). This compound is insoluble in water but is readily soluble in alcohol.

On being heated with acetic anhydride–pyridine on the steam-bath for 3 hours, the acid or its sodium salt gave rise to *O*-acetyleburicoic acid which separated from dilute alcohol in long silky needles, m. p. 256—257°, $[\alpha]_{\mathrm{D}}^{25}$ +33·6° (*c*, 3·0 in pyridine), $[\alpha]_{\mathrm{D}}^{21}$ +48·1° (*c*, 2·3 in chloroform), identical with the natural compound (Found : C, 76·7; H, 10·4. Calc. for $C_{32}H_{50}O_4$: C, 77·0; H, 10·1%).

Prepared with ethereal diazomethane, methyl eburicoate crystallised from methanol in slender needles, m. p. 140—141°, $[\alpha]_{\mathrm{D}}^{22}$ +33·1° (*c*, 3·4 in pyridine), $[\alpha]_{\mathrm{D}}^{17}$ +42·4° (*c*, 2·7 in chloroform) (Found : C, 78·6; H, 11·0. Calc. for $C_{31}H_{50}O_3$: C, 79·1; H, 10·7%). The methyl ester was also obtained by heating sodium eburicoate with methanolic methyl iodide for several hours and was isolated from the evaporated reaction mixture with benzene. This ester, which could not be prepared by the Fischer–Speier method, did not appear to be readily hydrolysed on being boiled with 10% alcoholic potassium hydroxide; after treatment for 2 hours 96% of the unchanged ester was recovered with only a trace of acid.

The acetate of methyl eburicoate separated from methanol in silky needles, m. p. 154—155°, $[\alpha]_{\mathrm{D}}^{22}$ +30·8° (*c*, 3·1 in pyridine), $[\alpha]_{\mathrm{D}}^{19}$ +48·7° (*c*, 2·4 in chloroform) (Found : C, 77·3; H, 10·1. Calc. for $C_{33}H_{52}O_4$: C, 77·3; H, 10·2%).

On being heated under reflux for 2 hours a mixture of sodium eburicoate (0·25 g.) and *p*-nitrobenzyl bromide (0·12 g.) in alcohol (15 ml.) gave rise to p-*nitrobenzyl eburicoate* which crystallised from alcohol in long colourless needles, m. p. 150—150·5° (Found : C, 74·8; H, 9·2; N, 2·5. $C_{37}H_{53}O_5N$ requires C, 75·0; H, 9·0; N, 2·4%).

(b) *From* Polyporus eucalyptorum *Fr*. The acid (yield 20%) had m. p. 293°, $[\alpha]_{\mathrm{D}}^{17}$ +35·6° (*c*, 1·4 in pyridine) (Found, C, 79·0, 78·8; H, 10·6, 10·6%), and gave the methyl ester, m. p. 140—141° (Found : C, 79·1; H, 10·5%), the *p*-nitrobenzyl ester, m. p. 150° (Found : C, 75·2; H, 8·9; N, 2·4%), and the acetate, m. p. 254°, $[\alpha]_{\mathrm{D}}^{25}$ +35·8° (*c*, 3·1 in pyridine) (Found : C, 77·0; H, 10·0%). Methyl *O*-acetyleburicoate had m. p. 153—154° (Found : C, 77·5; H, 10·3%).

(c) *From* Polyporus sulphureus (*Bull*) *Fr*. The acid (yield 10%) had m. p. 293°, $[\alpha]_{\mathrm{D}}^{20}$ +38·6° (*c*, 5·1 in pyridine) (Found : C, 79·1; H, 10·4%), and gave the acetate, m. p. 253—255°, $[\alpha]_{\mathrm{D}}^{19}$ +33·8° (*c*, 4·6 in pyridine) (Found : C, 76·8; H, 10·5%), the methyl ester, m. p. 140—141° (Found : C, 78·9; H, 10·8%), and the acetate of the methyl ester, m. p. 154—155° (Found : C, 77·1; H, 10·4%).

(d) *From* Fomes officinalis *Fr*. The crystalline acidic product (yield 6%) from this mould had m. p. 293° and gave an acetate, m. p. 254°, but differed from eburicoic acid from the sources (*a*), (*b*), and (*c*) in showing absorption in the ultra-violet (maximum at 243 mμ., $E^{1\%}_{1\,\mathrm{cm.}}$ 37·8). The acetate (6·45 g.) of this product was chromatographed from benzene–light petroleum (b. p. 60—80°) (1 l. of 1 : 4 mixture) on a column of alumina (60 cm. × 6 cm.; 1300 g.) which had been neutralised by being washed with methanol containing 10% of acetic acid and then several times with methanol, and dried at 120°. The column was eluted with benzene (3 l.), which removed only a trace of material, and then with 10% acetone in benzene. This gave a fraction (0·24 g.), $E^{1\%}_{1\,\mathrm{cm.}}$ 8·5 at 243 mμ., consisting of *O*-acetyleburicoic acid, which on recrystallisation from dilute alcohol had m. p. 256—257°, $[\alpha]_{\mathrm{D}}^{25}$ +31·9° (*c*, 2·2 in pyridine) (Found : C, 76·9; H, 10·4%). With ethereal diazomethane it gave methyl *O*-acetyleburicoate, m. p. 154—155° (Found : C, 76·9; H, 10·5%), and on hydrolysis furnished eburicoic acid, m. p. 292—293°, $[\alpha]_{\mathrm{D}}^{22}$ +35·1° (*c*, 1·6 in pyridine) (Found : C, 78·9; H, 10·7%), giving the methyl ester, m. p. 140° (Found : C, 78·8; H, 11·2%), and the *p*-nitrobenzyl ester, m. p. 151—152° (Found : C, 74·8; H, 9·1; N, 2·7%). Subsequent elution of the chromatogram with 50% acetone in benzene gave a series of fractions, of increasing $E^{1\%}_{1\,\mathrm{cm.}}$, which have been reserved for detailed examination.

(e) *From* Lentinus dactyloides *Clel*. The acidic fraction (yield *ca.* 5%) from the mycelium of this mould had m. p. 285° after repeated recrystallisation, with $E^{1\%}_{1\,\mathrm{cm.}}$ at 243 mμ. ranging from 99 to 125 in different batches. The mixed acetates (7 g.) were chromatographed on neutralised alumina [see (*d*)] (112 cm. × 1·8 cm.; 280 g.) from 700 ml. of 1 : 4-benzene–light petroleum (b. p. 60—80°), and the column was washed with a further 300 ml. of mixed solvent and allowed to drain. The filtrate contained only minute traces of material. The column was then divided into sections and each section eluted with hot methanol (250 ml. × 10), and the extracts evaporated. The bottom 5-cm. portion gave only a trace of material. The next and the subsequent 5-cm. portion gave solid products (0·12 g., $E^{1\%}_{1\,\mathrm{cm.}}$ 6·3 at 243 mμ., and 0·24 g., $E^{1\%}_{1\,\mathrm{cm.}}$ 6·6 at 243 mμ., respectively) which were combined and on recrystallisation from dilute alcohol gave *O*-acetyleburicoic acid, m. p. 255—256°, $[\alpha]_{\mathrm{D}}^{23}$ +36·4° (*c*, 1·7 in pyridine) (Found : C, 76·8; H, 10·3%), which was converted into methyl *O*-acetyleburicoic acid, m. p. 154—155° (Found : C, 76·8; H, 10·4%). On deacetylation the acetate gave eburicoic acid, m. p. 292—293°, $[\alpha]_D^{22}$ +34·8° (*c*, 1·5 in pyridine) (Found : C, 78·9; H, 10·4%), which formed the methyl ester, m. p. 140° (Found : C, 78·8; H, 11·0%), and the *p*-nitrobenzyl ester, m. p. 151—152° (Found : C, 75·3; H, 8·9; N, 2·6%).

The remaining sections of the column yielded products in which the value of $E_{1\,\text{cm.}}^{1\%}$ at 243 mμ. increased to a value of 110—115 at the top of the column.

The equivalent weight of eburicoic acid was determined by the procedure described by Ruzicka and Furter (*Helv. Chim. Acta*, 1932, **15**, 472), and the alkali employed was standardised against oleanolic acid. Thus eburicoic acid from *Polyporus anthracophilus* Cooke had equiv. 459, 462, from *P. eucalyptorum* Fr., equiv. 459, 461, and from *P. sulphureus* (Bull) Fr., equiv. 463, 465 (theoretical for $C_{30}H_{48}O_3$: equiv. 457). Betulic acid gave equiv. 457, 463 by same method.

Dihydroeburicoic Acid.—Hydrogenation of eburicoic acid (1·2 g.), dissolved in alcohol (250 ml.), with hydrogen (approx. 1 mol. absorbed) at atmospheric pressure and a palladium–charcoal catalyst (from 0·1 g. of palladium chloride and 1 g. of charcoal) was complete in one hour. On isolation the resulting *dihydroeburicoic acid* crystallised from alcohol in long needles, m. p. 295—297° (Found : C, 78·5; H, 10·9. $C_{30}H_{50}O_3$ requires C, 78·4; H, 11·0%). The *acetate* separated from dilute alcohol in long silky needles, m. p. 272—274°, giving a yellow coloration with tetranitromethane in chloroform (Found : C, 76·9; H, 10·4. $C_{32}H_{52}O_4$ requires C, 76·8; H, 10·5%), and the *methyl* ester from methanol in glistening prisms, m. p. 132° (Found : C, 78·6; H, 11·0. $C_{31}H_{52}O_3$ requires C, 78·7; H, 11·1%). *Methyl* O-*acetyldihydroeburicoate* formed stout prisms, m. p. 157—159° (Found : C, 76·9; H, 10·6. $C_{33}H_{54}O_4$ requires C, 76·9; H, 10·6%). Attempts to prepare a tetrahydro-derivative of eburicoic acid by means of an active platinum catalyst and hydrogen at 150°/100 atmospheres' pressure gave only the dihydro-compound.

Ozonolysis of O-*Acetyleburicoic Acid.*—A stream of ozone and oxygen was led into a solution of the acetate (1 g.) in chloroform (100 ml.) at 0° for 4 hours. After the evaporation of the solvent in a vacuum at room temperature the vitreous residue was treated with water (200 ml.), and 12 hours later the mixture was warmed on the steam-bath for ½ hour. Formaldehyde was removed from the reaction mixture by a current of steam, giving the dimedone derivative in needles, m. p. 189°, identified by comparison with an authentic specimen; the yield in several experiments ranged from 0·17 g. to 0·24 g., *i.e.*, 29% to 41% of the theoretical for one methylene group. In another experiment the formaldehyde was converted into the 2 : 4-dinitrophenylhydrazone, m. p. and mixed m. p. 162° after purification. In addition to formaldehyde, a colourless amorphous solid was obtained which gave an amorphous dinitrophenylhydrazone.

Ozonolysis of *O*-acetyldihydroeburicoic acid (1 g.) under the same conditions gave rise to only a trace (11 mg.) of the dimedone derivative of formaldehyde.

Oxidation of Methyl O-*Acetyleburicoate with Osmium Tetroxide.*—The oxide (1 g.) was added to a solution of the ester (3·72 g.) in ether (70 ml.), and on being kept for 24 hours the mixture deposited the osmic ester as a black solid. A solution of this product (2·27 g.), which had been washed with ether until the washings were colourless, in methanol (200 ml.) was added to aqueous sodium sulphite (30 g. in 150 ml. of water), and the mixture was heated under reflux for one hour and filtered. The solid was extracted with boiling methanol (3 × 100 ml.) and the combined filtrates and washings were evaporated in a vacuum. On concentration a methanolic extract of the residue gave a *diol* in small prisms (1·18 g.) which on recrystallisation from methanol had m. p. 195—196·5° (Found : C, 72·2; H, 10·2. $C_{33}H_{54}O_6$ requires C, 72·4; H, 9·9%).

Treatment of this diol (963 mg.), dissolved in acetic acid (8 ml.), with lead tetra-acetate (915 mg.) for 24 hours gave a *ketone*, part of which separated, the remainder being precipitated with water (50 ml.). On recrystallisation from methanol this compound formed long glistening needles (536 mg.), m. p. 177—178°; absorption spectrum $\lambda_{\text{max.}}$ 280 mμ., log ε 1·84 (Found : C, 74·6; H, 9·7. $C_{32}H_{50}O_5$ requires C, 74·6; H, 9·8%). The *oxime* separated from methanol in stout prisms, m. p. 171—172° (Found : C, 72·5; H, 9·8; N, 2·7. $C_{32}H_{51}O_5N$ requires C, 72·5; H, 9·7; N, 2·6%), and the *semicarbazone* in prisms, m. p. 218—219° (decomp.), absorption spectrum $\lambda_{\text{max.}}$ 225 mμ., log ε 4·18 (Found : C, 69·5; H, 9·5; N, 7·7. $C_{33}H_{53}O_5N_3$ requires C, 69·2; H, 9·3; N, 7·3%).

Treatment of the aqueous acetic acid filtrate from the crude ketone with dimedone gave the formaldehyde–dimedone derivative (360 mg.), m. p. and mixed m. p. 189°.

Oppenauer Oxidation of Eburicoic Acid.—A mixture of the acid (10 g.), *cyclo*hexanone (25 ml., 10 mol.), aluminium *tert.*-butoxide (15 g., 2·5 mol.), and dioxan (75 ml.) was heated under reflux for 6 hours, cooled, and poured into 2N-sulphuric acid (100 ml.). The dioxan and excess of *cyclo*hexanone were removed with steam, and the residual solid was crystallised from alcohol, giving *eburicodienonic acid* (8·1 g.) in needles, m. p. 226—227°, $[\alpha]_D^{20}$ +50·9° (*c*, 4·4 in pyridine); the absorption spectra showed an inflexion at 282 mμ. (log ε 1·54) (Found : C, 79·2; H, 10·2. $C_{30}H_{46}O_3$ requires C, 79·1; H, 10·2%). This acid, which is readily soluble in the usual solvents except light petroleum, did not react with Fehling's or Tollens's reagent. The *semicarbazone* separated from alcohol in leaflets, m. p. 313° (decomp.) (absorption spectrum max. at 225 mμ., log ε 4·17) (Found : C, 72·7; H, 9·5; N, 8·3. $C_{31}H_{49}O_3N_3$ requires C, 72·6; H, 9·6; N, 8·2%), and the *oxime* in needles, m. p. 243° (decomp.) (Found : C, 76·8; H, 10·2; N, 3·0. $C_{30}H_{47}O_3N$ requires C, 76·6; H, 10·1; N, 3·0%).

Eburicenonic acid was prepared from dihydroeburicoic acid by the method employed for eburicodienonic acid and formed rosettes of needles, m. p. 238—240° (Found : C, 79·0; H, 10·6. $C_{30}H_{48}O_3$

requires C, 78·9; H, 10·6%). The *semicarbazone* separated from alcohol in needles, m. p. 308—309° (decomp.) (Found : C, 72·5; H, 10·1; N, 8·0. $C_{31}H_{51}O_3N_3$ requires C, 72·4; H, 10·0; N, 8·2%).

Eburicodienic Acid.—A mixture of eburicodienonic acid (1·52 g.), 90% hydrazine hydrate (0·5 ml.), potassium hydroxide (0·7 g.), and diethylene glycol (5 ml.) was heated under reflux for 1½ hours. After the removal of the excess of hydrazine hydrate by distillation the reaction mixture was kept at 195° for 4 hours to decompose the hydrazone (evolution of gas and frothing), cooled, diluted with water (5 ml.), and treated with 6N-hydrochloric acid (6 ml.) followed by ether (2 ml.). A solid product separated and, after the evaporation of the ether, this was collected and crystallised from alcohol, giving *eburicodienic acid* in sheaves of needles (1·1 g.), m. p. 252—253°, $[\alpha]_D^{16}$ +40·5° (*c*, 3·0 in pyridine) (Found : C, 81·8; H, 11·1. $C_{30}H_{48}O_2$ requires C, 81·7; H, 11·0%). Prepared by use of ethereal diazomethane, the *methyl* ester formed needles, m. p. 98—99°, from methanol–chloroform (Found : C, 81·6; H, 11·2. $C_{31}H_{50}O_2$ requires C, 81·8; H, 11·1%), and on hydrogenation with W5 Raney nickel catalyst and hydrogen at atmospheric pressure gave rise to *methyl eburicenate* which crystallised from methanol–chloroform in elongated plates, m. p. 118—119·5° (Found : C, 81·6; H, 11·6. $C_{31}H_{52}O_2$ requires C, 81·4; H, 11·5%).

Eburicenol.—A mixture of methyl eburicenate (5·2 g.), lithium aluminium hydride (3 g.), and ether (150 ml.) was heated under reflux for 3 hours, kept for 14 hours, and poured on crushed ice (200 g.) and 2N-sulphuric acid (50 ml.). After the vigorous reaction had subsided the ethereal layer was isolated, washed, dried, and evaporated, leaving *eburicenol* (4·8 g.) which separated from methanol in rosettes of needles, m. p. 100—102°, $[\alpha]_D^{18}$ +54·5° (*c*, 3·0 in pyridine) (Found : C, 83·7; H, 12·3. $C_{30}H_{52}O$ requires C, 84·0; H, 12·2%). The *acetate* formed needles, m. p. 77—78°, from aqueous alcohol (Found : C, 81·7; H, 12·2. $C_{32}H_{54}O_2$ requires C, 81·6; H, 11·6%), and the *p-nitrobenzoate* needles, m. p. 112—114°, from methanol (Found : C, 76·5; H, 9·6. $C_{37}H_{55}O_4N$ requires C, 76·9; H, 9·6%).

Eburicenal.—A solution of eburicenol (3·8 g.), *p*-benzoquinone (8·8 g.), and aluminium *tert.*-butoxide (6 g.) in benzene (250 ml.) was heated under reflux for 14 hours. 2N-Sulphuric acid (50 ml.) was added to the cooled mixture, and the benzene and excess of quinone removed with steam. An ethereal solution of the reaction product was well washed with 2N-aqueous sodium hydroxide to remove the brown material and then with water, and dried, and evaporated, leaving a buff solid (3·2 g.); crystallised from alcohol–benzene, this gave *eburicenal* in colourless plates, m. p. 157—158°, $[\alpha]_D^{23}$ +53·1° (*c*, 3·0 in pyridine) (Found : C, 84·2; H, 12·0. $C_{30}H_{50}O$ requires C, 84·4; H, 11·8%). The *semicarbazone* separated from alcohol in short stout needles, m. p. 257—258° (decomp.) (Found : C, 76·9; H, 11·2; N, 8·8. $C_{31}H_{53}ON_3$ requires C, 76·9; H, 11·0; N, 8·7%).

Reduction of Eburicenal.—(*a*) A mixture of the semicarbazone (930 mg.) of eburicenal in alcohol (40 ml.), containing sodium ethoxide (from 1·8 g. of sodium), was kept at 190—200° for 12 hours, cooled, diluted with water, and acidified with dilute sulphuric acid. Isolated with ether, the product was a yellow oil (800 mg.) which was resolved into a main fraction (640 mg.), a colourless oil which solidified, and small amounts (80 mg. and 20 mg.) of bright yellow oils, by chromatography on alumina from light petroleum (b. p. 60—80°). On recrystallisation from alcohol–benzene, the solid fraction gave *eburicene*-I in colourless plates, m. p. 122—123°, $[\alpha]_D^{18}$ +55·0° (*c*, 2·0 in chloroform) (Found : C, 87·4; H, 12·6. $C_{30}H_{52}$ requires C, 87·3; H, 12·7%).

(*b*) A solution of eburicenal (770 mg.), potassium hydroxide (700 mg.), 90% hydrazine hydrate (0·5 ml.), and diethylene glycol (5 ml.) was heated under reflux for 1½ hours. The excess of hydrazine was removed by distillation and the mixture then kept at 195° for 4 hours, cooled, diluted with water, and acidified with dilute sulphuric acid. On isolation with ether the product was a yellow oil (600 mg.) which was resolved by chromatography from light petroleum (b. p. 60—80°) on alumina into two main and two subsidiary fractions. Fraction (1) (280 mg.) consisted of eburicene-I, m. p. and mixed m. p. 122—123° after recrystallisation from alcohol–benzene (Found : C, 87·3; H, 12·8%). Fraction (2) consisted of a colourless solid which on recrystallisation from alcohol–benzene gave *eburicene*-II in felted needles, m. p. 274—275°, $[\alpha]_D^{20}$ +51·3° (*c*, 0·8 in chloroform) (Found : C, 87·2, 87·1; H, 12·7, (12·7%. Fractions (3) (20 mg.) and (4) (100 mg.) were yellow oils which were not investigated.

Methyl Eburicotrienate.—A mixture of methyl eburicoate (1 g.), phosphorus pentachloride (0·75 g.), and light petroleum (b. p. 40—60°) (150 ml.) was vigorously agitated for 45 minutes and then kept for 24 hours. The light petroleum solution was washed with water (2 × 50 ml.), 2N-aqueous sodium hydroxide (2 × 50 ml.), and again with water (2 × 50 ml.), and dried and evaporated. Crystallisation of the residue from methanol gave *methyl eburicotrienate* in fine needles which in contact with the solvent changed to prisms (0·5 g.), m. p. 115—116° (Found : C, 82·6; H, 10·6. $C_{31}H_{48}O_2$ requires C, 82·2; H, 10·7%).

By the same method methyl dihydroeburicoate was converted into *methyl eburicodienate*-II which formed rectangular plates, m. p. 137—138°, from methanol (Found : C, 81·9; H, 10·9. $C_{31}H_{50}O_2$ requires C, 81·8; H, 11·1%).

Oxidation of Methyl Eburicodienate-II.—A mixture of methyl eburicodienate (696 mg.), osmium tetroxide (426 mg.), and ether (10 ml.) was kept for 24 hours, and the resulting black solid washed with cold ether until the washings were colourless. A solution of the residue (877 mg.) in methanol (50 ml.) was treated with sodium sulphite (10 g.; dissolved in 50 ml. of water), heated under reflux for 1 hour, and then filtered and the solid washed with methanol (50 ml.). The combined filtrate and washings were evaporated and the residue extracted with boiling methanol. On concentration the extract gave the *diol* in needles (361 mg.), m. p. 158—159°, after recrystallisation from methanol (Found : C, 76·3; H, 10·7. $C_{31}H_{52}O_4$ requires C, 76·2; H, 10·7%).

Oxidation of this diol (800 mg.) with lead tetra-acetate (800 mg.) in acetic acid (7 ml.) during 12 hours followed by dilution of the reaction mixture with water gave rise to a gum which did not crystallise. This product gave a *semicarbazone* forming, from methanol, rosettes of needles, m. p. 250°; light absorption, maximum 223 mμ., log ε 4·25 (Found : C, 71·8; H, 9·8; N, 8·8. $C_{29}H_{47}O_3N_3$ requires C, 71·7; H, 9·8; N, 8·7%). The 2 : 4-*dinitrophenylhydrazone* was purified by chromatography from benzene on alumina and then by crystallisation from methanol, forming orange needles, m. p. 225° (decomp.) (Found : C, 66·5; H, 8·1; N, 9·6. $C_{34}H_{48}O_6N_4$ requires C, 67·1; H, 8·0; N, 9·2%). Attempts to crystallise specimens of the ketone regenerated from the semicarbazone or from Girard reagent P were unsuccessful.

Acetone was isolated by distillation from the aqueous acetic acid liquor left after the separation of the crude ketone and was converted into the 2 : 4-dinitrophenylhydrazone, m. p. 125—126°, after purification from alcohol; yield, 70% of theoretical (Found : C, 45·5; H, 4·3; N, 23·4. Calc. for $C_9H_{10}O_4N_4$: C, 45·4; H, 4·2; N, 23·6%).

UNIVERSITY OF LIVERPOOL.

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