# 377. *The Chemistry of Fungi. Part XVII.* Dehydroeburicoic Acid.*

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Eburicoic acid is considered to have the empirical formula $C_{31}H_{50}O_3$.

The unsaturated acid with a conjugated diene system (Part XVI *) present in the mixed metabolic product of *Lentinus dactyloides* Clel. has been shown to be dehydroeburicoic acid by the oxidation of the mixture and isolation of dehydroeburiconic acid, identical with a specimen prepared from eburicoic acid by standard procedure. The epimer of dehydroeburicoic acid has also been prepared.

Experiments on the oxidation of the ring system of *O*-acetyleburicoic acid and a number of its derivatives are described. The similarities of eburicoic and dehydroeburicoic acid to lanosterol and agnosterol respectively are discussed.

From the results of the analysis of eburicoic acid and numerous derivatives (present work and unpublished observations) the compound is now considered to have the formula $C_{31}H_{50}O_3$, and not $C_{30}H_{48}O_3$ suggested earlier (*Nature*, 1951, **167**, 652; cf. Lahey and Strasser, *J.*, 1951, 873). This is supported by the molecular weight of methyl *O*-acetyldihydroeburicoate which was determined for us, through the kindness of Professor F. E. King of Nottingham, by Dr. S. C. Wallwork, using the *X*-ray method (Found: *M*, 526. Calc. for $C_{34}H_{56}O_4$: *M*, 529).

During the isolation of eburicoic acid from five species of fungi belonging to the class Basidiomycetes grown on a synthetical medium (Part XVI *) it was observed that in two species, *Lentinus dactyloides* Clel. and *Fomes officinalis* Fr., the acid was accompanied by a conjugated compound, having an absorption maximum at 243 mμ with subsidiary peaks at 236 and 251 mμ. The resolution of the mixtures proved difficult and, although small amounts of eburicoic acid could be separated by chromatography, the conjugated compound was not isolated. Other methods of separation have been explored, including crystallisation of various amine salts of the mixed acids from a variety of solvents, a technique which was successful with diterpene acids (Harris *et al.*, *J. Amer. Chem. Soc.*, 1948, **70**, 334, 2079, 3671, 3674) but in the present instance gave only a partial separation. The conjugated compound did not react with maleic anhydride even under the conditions in which the conjugated system of abietic acid ($\lambda_{max.}$ 241 mμ) is isomerised to form the adduct of lævopimaric acid (Bacon and Ruzicka, *Chem. and Ind.*, 1936, **55**, 546). Thus the acidic complexes appeared to resemble the mixture of lanosterol and dihydrolanosterol, and of agnosterol and dihydroagnosterol, which could be resolved only by oxidation with chromic anhydride to a mixture of ketones, separable by fractional crystallisation (Ruzicka, Denss, and Jeger, *Helv. Chim. Acta*, 1945, **28**, 759; 1946, **29**, 204). Consequently an oxidation procedure was applied to the product from *Lentinus dactyloides* and by the Oppenauer method this gave a mixture of keto-acids from which a spectroscopically pure compound [$\lambda_{max.}$ 243 mμ (log ε 4·24)] was isolated as the less soluble component by a lengthy fractional crystallisation. The similarity of the ultra-violet absorption of this compound to that of agnosterol, together with other considerations enumerated below, suggested that the conjugated compound co-existent with eburicoic acid in the fungi might be a dehydroeburicoic (eburicotrienolic, Part XVI) acid and consequently methods of preparation of a compound of this type from eburicoic acid were investigated. For this purpose three principal methods appeared feasible, *viz.*, dehydrogenation by selenium dioxide or *N*-bromosuccinimide, and oxidation by chromic acid to an αβ-unsaturated ketone capable of being reduced to an allyl alcohol which would be readily dehydrated to give the conjugated system. Whereas selenium dioxide reacted readily with *O*-acetyldihydroeburicoic acid, giving *O*-acetyldehydrodihydroeburicoic acid ($\lambda_{max.}$ 243 mμ, log ε 4·25) in good yield, *O*-acetyleburicoic acid, under similar conditions, formed intractable

* Part XVI, *J.*, 1951, 2346.

products owing to simultaneous attack at the reactive double bond. Similarly, with *N*-bromosuccinimide under a variety of conditions *O*-acetyleburicoic acid formed complex mixtures. More success was achieved with the third method. Oxidation of *O*-acetyleburicoic acid with chromic anhydride under relatively mild conditions led to the introduction of carbonyl groups with the formation of only small amounts of by-products. On deacetylation the main reaction product was found to be a difficultly separable mixture of colourless ketoeburicoic acid ($\lambda_{max.}$ 253 mμ, log ε 3·97), and yellow diketoeburicoic acid ($\lambda_{max.}$ 270 mμ, log ε 3·90). As the required monoketo-acid appeared to be readily oxidised to the diketo-compound the maximum yield of the required ketoeburicoic acid was obtained with conditions where some eburicoic acid was recovered unchanged. Reduction of the monoketo-acid with sodium in boiling amyl alcohol led directly to dehydroeburicoic acid and the intermediate hydroxyeburicoic acid was not isolated. Thus obtained, dehydroeburicoic acid had $\lambda_{max.}$ 243 mμ (log ε 4·24) and on oxidation by the Oppenauer method yielded dehydroeburiconic acid which, by comparison of their methyl esters, infra-red absorption spectra, and other physical properties, was found to be identical with the keto-acid separated from the product formed by the Oppenauer oxidation of the mixed acids isolated from *Lentinus dactyloides*. The conjugated hydroxy-acid produced by this mould along with eburicoic acid is, therefore, either dehydroeburicoic acid or its epimer. The latter possibility has been excluded by a comparison with the corresponding *epi*-compounds which can be separated readily from their respective isomerides and, therefore, since one component of the mixed acetylated acids from *Lentinus dactyloides* Clel. is *O*-acetyleburicoic acid (Part XVI) the other is clearly *O*-acetyldehydroeburicoic acid.

The production of eburicoic and dehydroeburicoic acid by fungi is analogous to the occurrence of agnosterol and lanosterol in wool wax. The wool-wax mixture also contains dihydrolanosterol and dihydroagnosterol (Ruzicka, Rey, and Muhr, *Helv. Chim. Acta*, 1944, **27**, 472) but no evidence has been obtained indicating the presence of dihydro-compounds in the fungal mixtures. The comparisons enumerated below show that eburicoic and dehydroeburicoic acids are very similar in many respects to lanosterol and agnosterol respectively, and provide circumstantial evidence that the structural residues (I) and (II) of the last-named compounds are present in eburicoic and dehydroeburicoic acid respectively.

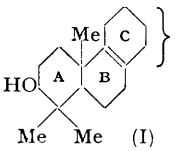
(1) *O*-Acetyldihydroeburicoic acid does not exhibit infra-red absorption in the 12-μ region that could be attributed to a carbon–carbon double bond, thus indicating that the double bond present in the molecule is tetrasubstituted. The ultra-violet absorption spectra of dehydroeburicoic acid and agnosterol are very similar whilst the infra-red absorption of *O*-acetyldehydrodihydroeburicoic acid exhibits a peak at 813 cm.$^{-1}$, and Voser, Montavon, Günthard, Jeger, and Ruzicka (*Helv. Chim. Acta*, 1950, **33**, 1893) record that dihydroagnosterol acetate has strong absorption in the infra-red at 814 cm.$^{-1}$ which they ascribe to the trisubstituted double bonds.

(2) Reduction of *O*-acetyldehydroeburicoic acid with Adams' catalyst at room temperature and atmospheric pressure or at 30 atmospheres produced *O*-acetyldehydrodihydroeburicoic acid ($\lambda_{max.}$ 243 mμ, log ε 4·26), identical with the product formed by selenium dioxide oxidation of *O*-acetyldihydroeburicoic acid, in which only the exocyclic double bond has been reduced. Similarly, Birchenough and McGhie (*J.*, 1949, 2038) record that the two nuclear double bonds of dihydroagnosterol cannot be hydrogenated (cf. also Ruzicka *et al.*, *loc. cit.*, 1946).

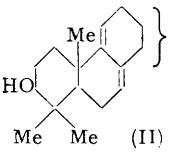
(3) The formation of keto- and diketo-eburicoic acid by the chromic anhydride oxidation of *O*-acetyleburicoic acid is in accordance with the properties of tetracyclic triterpenes in general (cf. Haines and Warren, *J.*, 1950, 1562). For the mono- and di-keto-compounds thus formed from dihydrolanosterol the partial formulæ (III) and (IV) have been established (Voser *et al.*, *loc. cit.*; see also Barton, Fawcett, and Thomas, *J.*, 1951, 3147).

The oxidation of *O*-acetyldehydrodihydroeburicoic acid and *O*-acetyldihydroeburicoic acid with chromic anhydride yielded *O*-acetyldihydrodiketoeburicoic acid, the methyl ester of which has been described by Lahey and Strasser (*J.*, *loc. cit.*). Similarly, oxidation of dihydroagnosteryl acetate and of dihydrolanosteryl acetate, respectively, yielded the

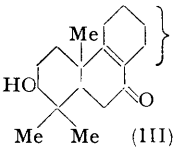
same dihydrodiketolanosteryl acetate (Dorée, McGhie, and Kurzer, *J.*, 1948, 988). Reduction of methyl *O*-acetyldihydrodiketoeburicoate with zinc and acetic acid yielded colourless methyl acetyltetrahydrodiketoeburicoate ($\lambda_{max.}$ 295 mμ, log ε 1·80), where the ene-1 : 4-dione chromophore has been reduced to a 1 : 4-diketonic system as in the conversion of dihydrodiketolanosteryl acetate into diketolanostanyl acetate (Dorée *et al.*, *loc. cit.*) having a structure of type (V) (Voser *et al.*, *loc. cit.*).



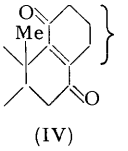
(I)



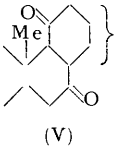
(II)



(III)



(IV)



(V)

The ultra-violet absorption spectra of keto- and diketo-eburicoic acid are similar to those of dihydroketolanosterol and dihydrodiketolanosterol respectively, and *O*-acetyldihydrodiketoeburicoic acid does not show absorption in the 12-μ region which could be attributed to a carbon–carbon double bond. Dihydrodiketolanosteryl acetate does not show ethylenic absorption in the infra-red and from this Voser *et al.* (*loc. cit.*) deduced that the ene-1 : 4-dione system must have the transoid arrangement shown in (IV) since normally double bonds conjugated with keto-groups have strong infra-red absorption. These considerations suggest that the structures (III), (IV), and (V) are applicable also to keto-, diketo-, and tetrahydrodiketo-eburicoic acid respectively.

(4) The methods which have been used to convert eburicoic and dihydroeburicoic acid into their respective dehydro-compounds are those used in the conversion of dihydrolanosterol into dihydroagnosterol, *viz.*, (*a*) reduction and dehydration of ketoeburicoic acid (cf. the reduction and dehydration of dihydroketolanosterol; Marker, Wittle, and Mixon, *J. Amer. Chem. Soc.*, 1937, **59**, 1368; Cavalla and McGhie, *J.*, 1951, 744) and (*b*) the action of selenium dioxide on *O*-acetyldihydroeburicoic acid (cf. Bellamy and Dorée, *J.*, 1941, 176; Dorée, McGhie, and Kurzer, *J.*, 1949, 570); the action of *N*-bromosuccinimide on *O*-acetyleburicoic acid gave an inseparable mixture. Further, Lahey and Strasser (*loc. cit.*) observed that with perbenzoic acid methyl *O*-acetyldihydroeburicoate gave a compound having $\lambda_{max.}$ 243 mμ (log ε 4·24), which they term methyl acetyl*iso*-eburicoate, and which is undoubtedly identical with methyl *O*-acetyldehydrodihydroeburicoate now obtained. As Lahey and Strasser indicated, the formation of this ester is analogous to the conversion of dihydrolanosteryl into dihydroagnosteryl acetate by perbenzoic acid (Birchenough and McGhie, *loc. cit.*).

In their comparatively ready conversion into dehydro-derivatives lanosterol and eburicoic acid can be differentiated from other tetracyclic triterpenes, *viz.*, euphol, tirucallol, euphorbol, and elemadienolic acid. Thus dihydrolanosteryl acetate and *O*-acetyldihydroeburicoic acid react readily with selenium dioxide in hot acetic acid to yield their respective dehydro-compounds under conditions where dihydroeuphyl acetate (Vilkas, Dupont, and Dulou, *Bull. Soc. chim.*, 1949, 813) and elemenolic acid (Ruzicka, Rey, and Spillmann, *Helv. Chim. Acta*, 1942, **25**, 1375) are not attacked. Similarly, the epoxides of dihydrolanosteryl acetate (Birchenough and McGhie, *loc. cit.*) and *O*-acetyldihydroeburicoic acid (Lahey and Strasser, *loc. cit.*) formed by perbenzoic acid are very unstable, yielding the dehydro-compounds virtually spontaneously, whereas the epoxides from dihydroeuphyl, dihydrotirucallyl, and dihydroeuphorbyl acetate require treatment with a dehydrating agent to convert them into the dehydro-compounds (Barbour, Bennett, and Warren, *J.*, 1951, 2540).

(5) The molecular-rotation differences observed on acetylation and oxidation of the tetracyclic triterpenes (Table 1; cf. Heilbron, Jones, and Robins, *J.*, 1949, 444) show considerable divergencies between most of the members of this group. These divergencies are in contrast to the uniformity of the values in the case of eburicoic acid and its derivatives compared with those of lanosterol and its corresponding derivatives. This similarity between eburicoic acid and lanosterol appears also in the molecular-rotation differences observed with the dehydro-compounds (Table 2) and with the ene-1 : 4-diones prepared from members of the tetracyclic group (Table 3).

TABLE 1.

| | $[M]_D$ | | | | | |
|---|---|---|---|---|---|---|
| | | Acetate | Ketone | ΔAc | ΔKet | Refs. to values of $[\alpha]_D$ † |
| Eburicoic acid | +235° | +249° | +248° | + 14° | + 13° | 1 |
| Dihydroeburicoic acid | +215 | +253 | — | + 36 | — | 1 |
| Methyl eburicoate | +222 | +256 | +287 | + 34 | + 65 | 1 |
| Methyl dihydroeburicoate | +219 | +270 | — | + 51 | — | 1 |
| Lanosterol | +258 | +313 | +333 | + 55 | + 75 | 2, 3 |
| Dihydrolanosterol | +250 | +278 | +318 | + 28 | + 68 | 2, 3, 4, 5, 6 |
| Euphol | +134 | +202 | +306 | + 68 | +172 | 7, 8, 9, 10, 32 |
| Dihydroeuphol | +131 | +165 | +277 | + 34 | +146 | 7, 8, 9 |
| *epi*Elemadienolic acid | + 43 | +130 | +205 | + 87 | +162 | 11, 12, 13, 14 |
| *epi*Elemenolic acid | + 69 | + 80 | +197 | + 11 | +128 | 11, 12, 13 |
| Elemadienolic acid | −110 | −200 | +205 | − 90 | +315 | 11, 12, 13, 14, 15, 16 |
| Elemenolic acid | − 83 | −160 | +197 | − 77 | +280 | 11, 12, 13, 14, 17, 18, 19 |
| Methyl elemadienolate | − 68 | −216 | +162 | −148 | +230 | 11, 12, 14 |
| Methyl elemenolate | − 28 | −185 | — | −157 | — | 11, 12, 14, 19 |
| Tirucallol | (+19) * | (−77) * | — | (− 96) | — | 20 |
| Dihydrotirucallol | (+13) * | (−55) * | +171 | (− 68) | (+158) | 20, 21 |
| Euphorbol | 0 | 0 | + 93 | 0 | + 93 | 7, 22 |
| Butyrospermol | − 53 | + 52 | −162 | +105 | −109 | 23, 24 |
| Dihydrobutyrospermol | − 56 | + 52 | −188 | +108 | −132 | 23, 24 |

* Measurement of $[\alpha]_D$ in benzene solution. † See Table 3.

TABLE 2.

| | $[M]_D$ | | | | | |
|---|---|---|---|---|---|---|
| | | Acetate | Ketone | ΔAc | ΔKet | Refs. to values of $[\alpha]_D$ * |
| Dehydroeburicoic acid | +188° | +317° | +131° | +129° | −59° | 1 |
| Dehydrodihydroeburicoic acid | +184 | +346 | +113 | +162 | −71 | 1 |
| Methyl dehydroeburicoate | +207 | +357 | +139 | +150 | −68 | 1 |
| Methyl dehydrodihydroeburicoate | +204 | +364 | +116 | +160 | −88 | 1 |
| Agnosterol | +283 | +414 | +212 | +131 | −71 | 25 |
| Dihydroagnosterol | +284 | +411 | +202 | +127 | −82 | 4, 5, 25, 26 |
| Dehydrodihydroeuphol (Dehydroeuphenol) | −508 | − 42 | — | +466 | — | 27 |
| Dehydrodihydrotirucallol (Dehydrotirucallenol) | −701 | −639 | — | + 62 | — | 27 |
| Dehydrodihydroeuphorbol (Dehydroeuphorbenol) | −581 | −652 | — | − 71 | — | 27 |

* See Table 3.

TABLE 3.

| | $[M]_D$ | | | | | |
|---|---|---|---|---|---|---|
| Ene-1 : 4-dione prepared from | | Acetate | Ketone | ΔAc | ΔKet | Refs. to values of $[\alpha]_D$ |
| Eburicoic acid | +314° | +395° | — | +81° | — | 1 |
| Dihydroeburicoic acid | +301 | +391 | +664° | +90 | +363° | 1 |
| Methyl dihydroeburicoate | +306 | +395 | +595 | +89 | +289 | 1 |
| Dihydrolanosterol | +356 | +454 | +787 | +98 | +431 | 4, 28, 29, 30 |
| Elemenolic acid | − 54 | −148 | + 37 | −94 | + 91 | 12, 31 |
| Dihydroeuphol | +128 | +114 | +141 | −14 | + 13 | 21, 32 |
| Dihydrotirucallol | −112 | − 85 | — | +27 | — | 21 |
| Dihydroeuphorbol | −270 | −200 | — | +70 | — | 22 |

[1] For eburicoic acid and its derivatives see Lahey and Strasser (*loc. cit.*), Part XVI (*loc. cit.*), and this communication. [2] Ruzicka, Denss, and Jeger, *Helv. Chim. Acta*, 1945, **28**, 759. [3] Wieland, Pasedach, and Ballauf, *Annalen*, 1937, **529**, 68. [4] Ruzicka, Rey, and Muhr, *Helv. Chim. Acta*, 1944, **27**, 472. [5] Windaus and Tschesche, *Z. physiol. Chem.*, 1930, **190**, 51. [6] Wieland and Benend, *ibid.*, 1942, **274**, 215. [7] Newbold and Spring, *J.*, 1944, 249. [8] Jeger and Krusi, *Helv. Chim. Acta*, 1947, **30**, 2045. [9] Roth and Jeger, *ibid.*, 1949, **32**, 1620. [10] Bennett and Warren, *J.*, 1950, 697. [11] Ruzicka and Hausermann, *Helv. Chim. Acta*, 1942, **25**, 439. [12] Ruzicka, Rey, Spillmann, and Baumgartner, *ibid.*, 1943, **26**, 1638. [13] Bilham and Kon, *J.*, 1942, 544. [14] Ruzicka, Rey, and Spillmann, *Helv. Chim. Acta*, 1942, **25**, 1375. [15] Lieb and Mladenovic, *Monatsh.*, 1932, **61**, 274. [16] *Idem*, *ibid.*, 1931, **58**, 59. [17] Ruzicka and Furter, *Helv. Chim. Acta*, 1932, **15**, 472. [18] Lieb and Mladenovic, *Monatsh.*, 1932, **59**, 228. [19] Ruzicka, Wakeman, Furter, and Goldberg, *Helv. Chim. Acta*, 1932, **15**, 1454. [20] Haines and Warren, *J.*, 1949, 2554. [21] *Idem*, *J.*, 1950, 1562. [22] Barbour, Warren, and Wood, *J.*, 1951, 2537. [23] Heilbron, Jones, and Robins, *J.*, 1949, 444. [24] Seitz and Jeger, *Helv. Chim. Acta*, 1949, **32**, 1626. [25] Ruzicka, Denss, and Jeger, *Helv. Chim. Acta*, 1946, **29**, 204. [26] Birchenough and McGhie, *J.*, 1949, 2038. [27] Barbour, Bennett, and Warren, *J.*, 1951, 2540. [28] Birchenough and McGhie, *J.*, 1950, 1249. [29] Cavalla and McGhie, *J.*, 1951, 744. [30] Cavalla and McGhie, *J.*, 1951, 834. [31] Ruzicka, Rey, Spillmann, and Baumgartner, *Helv. Chim. Acta*, 1943, **26**, 1659. [32] Christen, Dünnenberger, Roth, Heusser, and Jeger, *Helv. Chim. Acta*, 1952, **35**, 1756.

## Experimental

Specific rotations were measured in chloroform, 1-dm. tubes being used 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; 5—8% solutions of carbon disulphide and a 0·2-mm. cell were employed except where stated.

*Isolation of Dehydroeburiconic Acid from Mixed Acids of* Lentinus dactyloides *Clel.*—Aluminium *tert.*-butoxide (6·9 g.) was added to a solution of the mixed eburicoic and dehydroeburicoic acid (3·3 g.; $\lambda_{max.}$ 243 mμ, $E^{1\%}_{1cm.}$ 100) from the mycelium of *Lentinus dactyloides* Clel. in dioxan (28 ml.) containing *cyclo*hexanone (8 ml.), and the mixture, which became clear when warmed, was heated under reflux for 12 hr., cooled, and treated with an excess of dilute sulphuric acid. After the removal of the solvents in a current of steam the residual solid was isolated, washed with water, dried, and crystallised from alcohol (charcoal). The first fraction (1·13 g.) had m. p. 220—225°, $\lambda_{max.}$ 243 mμ, $E^{1\%}_{1cm.}$ 179, and the second (1·52 g.) had m. p. 218—225°, $\lambda_{max.}$ 243 mμ, $E^{1\%}_{1cm.}$ 90. Thus obtained, the two products were subjected to a prolonged fractional crystallisation from alcohol in which fractions having equal intensities of absorption at 243 mμ were combined. *Dehydroeburiconic acid* was ultimately obtained and, on repeated recrystallisation from alcohol, had a constant value for the absorption at points of maximum intensity; the yield was 130 mg. (no attempt was made to isolate eburiconic acid, named eburicodienonic acid in Part XVI, *loc. cit.*). This acid formed colourless needles, m. p. 241—242° (from alcohol), $\lambda_{max.}$ 234, 243, 252 mμ (log ε 4·19, 4·25, 4·08) and an inflexion at about 285 mμ (log ε 1·78), $[\alpha]_D^{20}$ +27·0° (*c*, 1·9, 0·5-dm. micro-tube) (Found: C, 79·8; H, 10·1. $C_{31}H_{46}O_3$ requires C, 79·8; H, 9·9%). Determined with a paste in "Nujol" the infra-red absorption spectra of this compound was identical with that of a specimen prepared from eburicoic acid. The *methyl* ester was prepared with ethereal diazomethane containing a trace of methanol and on purification from methanol formed needles, m. p. 159—160°, $[\alpha]_D^{16}$ +28·5° (*c*, 2·53 in 0·5-dm. micro-tube) (Found: C, 80·1; H, 10·3. $C_{32}H_{48}O_3$ requires C, 80·0; H, 10·1%).

*Oxidation of* O-*Acetyleburicoic Acid with Chromic Acid.*—A solution of *O*-acetyleburicoic acid (17 g.) in acetic acid (750 ml.) was treated with chromic anhydride (5·55 g.) dissolved in acetic acid (200 ml.) containing water (40 ml.), kept for 4 days, and diluted with water (10·0 l.). The resulting precipitate was boiled with 10% alcoholic potassium hydroxide (300 ml.) for 1 hr. and, after dilution with water (750 ml.), the hydrolysate was concentrated until the potassium salts began to separate. On isolation the resulting mixed salts were digested with boiling 0·1N-sodium hydroxide (2 l.), leaving the potassium salt of unchanged eburicoic acid (2·5 g.). Acidification of the cooled extract gave a mixture of keto- and diketo-eburicoic acid; more of this mixture (1·0 g.) was obtained by acidification of the concentrated liquor from the crude salts and boiling the product with 15% aqueous sodium carbonate, whereby a mixture of the insoluble sodium salts of the acids was obtained which was washed with aqueous sodium carbonate and decomposed with 2N-hydrochloric acid. Crystallised from aqueous alcohol, the mixed keto-acids formed light yellow slender needles (9·8 g.), m. p. 251—254°, $\lambda_{max.}$ 254—255 mμ. This product (2·7 g.) was acetylated with pyridine and acetic anhydride on the steam-bath, and a solution of the mixed acetates in benzene (200 ml.) was poured on a column of neutralised aluminium oxide (130 g.) which was then washed with benzene (1·2 l.) to remove mixed anhydro-compounds (0·61 g.), presumably formed during acetylation of the keto-acids. Elution with acetone–benzene (1 l.; 1 : 19) then gave O-*acetylketoeburicoic acid* (0·92 g.), $\lambda_{max.}$ 253 mμ, which formed colourless needles, m. p. 229—231°, $[\alpha]_D^{20}$ +18° (*c*, 2·5; 0·5-dm. micro-tube) (Found: C, 75·6; H, 9·4. $C_{33}H_{50}O_5$ requires C, 75·2; H, 9·6%), and on deacetylation yielded *ketoeburicoic acid*, separating from dilute alcohol in colourless needles, m. p. 253—254°, $[\alpha]_D^{21}$ +24° (*c*, 1·63; 0·5-dm. micro-tube), $\lambda_{max.}$ 253 mμ (log ε 3·97) with an inflexion at about 332 mμ (log ε 1·71) (Found, in specimen sublimed in a high vacuum: C, 76·7; H, 9·9. $C_{31}H_{48}O_4$ requires C, 76·8; H, 10·0%). After the elution of *O*-acetylketoeburicoic acid the column was washed with acetone–benzene (1 : 10, then 1 : 5) and gave a series of fractions the $\lambda_{max.}$ of which progressively increased. Finally, elution with acetone (1·3 l.) furnished O-*acetyldiketoeburicoic acid* (0·29 g.), which formed pale yellow needles, m. p. 279—280°, $[\alpha]_D^{21}$ +73° (*c*, 4·75; 0·5-dm. micro-tube), from dilute alcohol (Found: C, 73·4; H, 8·7. $C_{33}H_{48}O_6$ requires C, 73·3; H, 8·9%). On deacetylation this acetate gave *diketoeburicoic acid* which separated from dilute alcohol in pale yellow needles, m. p. 260—262°, $[\alpha]_D^{21}$ +63° (*c*, 1·1), $\lambda_{max.}$ 270, 337 mμ (log ε 3·90, 1·68) with an inflexion at about 405 mμ (log ε 1·40) (Found, in specimen sublimed in a high vacuum: C, 74·7; H, 9·2. $C_{31}H_{46}O_5$ requires C, 74·7; H, 9·3%).

*Dehydroeburicoic Acid.*—To a boiling solution (oil-bath) of ketoeburicoic acid (0·09 g.) in *n*-amyl alcohol sodium (1 g.) was added gradually during 1 hr., with more *n*-amyl alcohol to maintain a clear solution. The cooled mixture was treated with water followed by an excess of dilute hydrochloric acid, the amyl alcohol layer was isolated, and the alcohol removed with steam, leaving a pale yellow solid (0·08 g.), m. p. 255—265°, $\lambda_{max.}$ 234, 243, 252 mμ, $E^{1\%}_{1cm.}$ 202, 236, 161, which on being recrystallised five times from ethyl acetate gave *dehydroeburicoic acid* in slender needles, m. p. 286—288°, $[\alpha]_D^{23}$ +40° (*c*, 0·2; *l*, 4·0), $\lambda_{max.}$ 234, 243, 252 mμ, $E^{1\%}_{1cm.}$ 320, 375, 252 (Found: C, 79·1; H, 10·4. $C_{31}H_{48}O_3$ requires C, 79·4; H, 10·3%). *Methyl dehydroeburicoate* was prepared from the acid with ethereal diazomethane containing methanol, and on crystallisation from benzene–light petroleum and then aqueous methanol formed colourless needles, m. p. 146·5—147·5°, $[\alpha]_D^{23}$ +43° (*c*, 3·1; 0·5-dm. micro-tube) (Found: C, 79·6; H, 10·5. $C_{32}H_{50}O_3$ requires C, 79·6; H, 10·4%). By the pyridine–acetic anhydride method the acid gave O-*acetyldehydroeburicoic acid* which separated from alcohol in needles, m. p. 255—256°, $[\alpha]_D^{21}$ +62° (*c*, 2·4) (Found: C, 77·7; H, 10·0. $C_{33}H_{50}O_4$ requires C, 77·6; H, 9·9%). On admixture with *O*-acetyleburicoic acid it gave no depression in the m. p. Formed by means of ethereal diazomethane containing a trace of methanol, *methyl* O-*acetyldehydroeburicoate* formed needles, m. p. 159—160°, $[\alpha]_D^{23}$ +68° (*c*, 4·7; 0·5-dm. tube) from methanol (Found: C, 77·7; H, 10·1. $C_{34}H_{52}O_4$ requires C, 77·8; H, 10·0%).

*Dehydroeburiconic Acid from Dehydroeburicoic Acid.*—A mixture of dehydroeburicoic acid (0·5 g.), aluminium *tert.*-butoxide (1 g.), dioxan (4·25 ml.), and *cyclo*hexanone (1·15 ml.) was heated under reflux for 8 hr. and the resulting dehydroeburiconic acid was isolated in the usual manner by the method employed in the isolation of the compound from metabolic acids. Recrystallised from methanol, the acid formed needles (0·3 g.), m. p. 240—242°, $[\alpha]_D^{21}$ +28° (*c*, 0·8), $\lambda_{max.}$ 234, 243, 252 mμ (log ε 4·19, 4·26, 4·08) with an inflexion at about 285 mμ (log ε 1·79) (Found: C, 79·5; H, 10·1. Calc. for $C_{31}H_{46}O_3$: C, 79·8; H, 9·9%). Methyl dehydroeburiconate separated from methanol in needles, m. p. 159—160°, $[\alpha]_D^{21}$ +29·5° (*c*, 2·5; 0·5-dm. micro-tube) (Found: C, 80·0; H, 10·2. Calc. for $C_{32}H_{48}O_3$: C, 80·0; H, 10·1%).

*Dehydrodihydroeburicoic Acid.*—A solution of *O*-acetyldihydroeburicoic acid (2·7 g.), in 95% acetic acid (75 ml.), containing selenium dioxide (3·6 g.), was refluxed for 3 hr., filtered, and diluted with water. After having been crystallised from dilute alcohol, the product was chromatographed in benzene on a short column of neutralised aluminium oxide. The resulting O-*acetyldehydrodihydroeburicoic acid* (2·1 g.) separated from alcohol in colourless needles, m. p. 270—273°, $[\alpha]_D^{21}$ +65° (*c*, 2·5), $\lambda_{max.}$ 234, 243, 252 mμ (log ε 4·18, 4·25, 4·06) (Found: C, 77·0; H, 10·1. $C_{33}H_{52}O_4$ requires C, 77·3; H, 10·2%). The same compound (0·18 g.) was obtained by hydrogenation of *O*-acetyldehydroeburicoic acid (0·2 g.), dissolved in alcohol (25 ml.), with a platinum catalyst and hydrogen (1·05 mol. absorbed) at room temperature and pressure or at a pressure of 30 atm. Methyl *O*-acetyldehydrodihydroeburicoate formed needles, m. p. 164—164·5°, $[\alpha]_D^{20}$ +69° (*c*, 2·0), from methanol (Found: C, 77·1; H, 10·3. Calc. for $C_{34}H_{54}O_4$: C, 77·5; H, 10·3%) (cf. Lahey and Strasser, *loc. cit.*).

*O*-Acetyldehydrodihydroeburicoic acid (0·8 g.) was deacetylated with boiling 10% alcholic potassium hydroxide (50 ml.) during 3 hr. and the hydrolysate treated with water (25 ml.). On evaporation of the greater part of the alcohol potassium dehydrodihydroeburicoate separated which on decomposition in alcohol with hydrochloric acid gave the *acid*, separating from alcohol in slender needles, (0·65 g.), m. p. 298—300°, $[\alpha]_D^{20}$ +39° (*c*, 0·16; 4-dm. tube) (Found: C, 78·7; H, 10·8. $C_{31}H_{50}O_3$ requires C, 79·1; H, 10·7%). Formed by deacetylation of methyl *O*-acetyldehydrodihydroeburicoate the *methyl* ester formed needles, m. p. 162—163°, with softening at 148°, $[\alpha]_D^{20}$ +42° (*c*, 4·4; 0·5-dm. micro-tube), from methanol (Found: C, 79·3; H, 10·9. $C_{32}H_{52}O_3$ requires C, 79·3; H, 10·8%).

*Dehydrodihydroeburiconic Acid.*—Oxidation of dehydrodihydroeburicoic acid (0·5 g.) with aluminium *tert.*-butoxide (1 g.) and *cyclo*hexanone (1·15 ml.) in boiling dioxan (4·25 ml.) for 8 hr. gave *dehydrodihydroeburiconic acid* which separated from alcohol in needles, (0·3 g.), m. p. 252—254°, $[\alpha]_D^{21}$ +24° (*c*, 1·7) (Found: C, 79·5; H, 10·5. $C_{31}H_{48}O_3$ requires C, 79·4; H, 10·3%). The *methyl* ester crystallised from methanol as needles, m. p. 155—155·5°, $[\alpha]_D^{23}$ +24° (*c*, 0·9) (Found: C, 79·6; H, 10·4. $C_{32}H_{50}O_3$ requires C, 79·6; H, 10·4%).

*Dihydrodiketoeburicoic Acid.*—(*a*) Oxidation of *O*-acetyldihydroeburicoic acid (0·5 g.) in acetic acid (20 ml.) was effected with chromic anhydride (0·33 g.) in acetic acid (6 ml.) and water (1·2 ml.) at room temperature during 7 days, or at 80° for 1 hr. The solid obtained by dilution of the reaction mixture with water (450 ml.) was deacetylated with hot 10% alcoholic potassium hydroxide (50 ml.), and the resulting almost pure dihydrodiketoeburicoic acid isolated by way of the potassium salt and recrystallised from dilute alcohol having m. p. 274—276° (yield,

0·35 g.). This product was acetylated and the resulting O-*acetyldihydrodiketoeburicoic acid* purified by chromatography from benzene on neutralised aluminium oxide, and then crystallised from dilute alcohol, forming needles (0·25 g.), m. p. 286—288° (decomp.), $[\alpha]_D^{21}$ +72° (*c*, 2·1), $\lambda_{max.}$ 269, 332—334 mμ (log ε 3·97, 1·87) with an inflexion at about 405 mμ (log ε 1·6) (Found: C, 72·9; H, 9·5. $C_{33}H_{50}O_6$ requires C, 73·0; H, 9·3%). The methyl ester separated from methanol in thick yellow prisms, m. p. 172°, $[\alpha]_D^{22}$ +71° (*c*, 0·6), $\lambda_{max.}$ 269—270, 332—334 mμ (log ε 3·99, 1·89) with an inflexion at about 405 mμ (log ε 1·65) (Found: C, 73·6; H, 9·6. Calc. for $C_{34}H_{52}O_6$: C, 73·3; H, 9·4%) (cf. Lahey and Strasser, *loc. cit.*).

Deacetylation of *O*-acetyldihydrodiketoeburicoic acid gave *dihydrodiketoeburicoic acid* which crystallised from dilute alcohol in slender needles, m. p. 276—277°, $[\alpha]_D^{18}$ +60° (*c*, 0·6) (Found, in specimen sublimed in a high vacuum: C, 74·6; H, 9·7. $C_{31}H_{48}O_5$ requires C, 74·4; H, 9·7%). The *methyl* ester separated from methanol in yellow needles, m. p. 88°, containing solvent of crystallisation. Recrystallised from light petroleum (b. p. 60—80°), the compound formed yellow needles, m. p. 140—141°, $[\alpha]_D^{21}$ +59·5° (*c*, 1·42) (Found: C, 75·1; H, 10·0. $C_{32}H_{50}O_5$ requires C, 74·7; H, 9·8%).

(*b*) The oxidation of *O*-acetyldehydrodihydroeburicoic acid by method (*a*) gave a product from which *O*-acetyldihydrodiketoeburicoic acid was isolated by chromatography from benzene on neutralised aluminium oxide and on recrystallisation had m. p. and mixed m. p. 286—288° (decomp.), $[\alpha]_D^{21}$ +70° (*c*, 1·2), and the expected ultra-violet absorption spectrum (Found: C, 73·3; H, 9·4%). The methyl ester had m. p. and mixed m. p. 171·5—172°, $[\alpha]_D^{21}$ +69° (*c*, 1·2) (Found: C, 73·6; H, 9·6%). The ultra-violet and infra-red absorption spectra of the specimens of methyl *O*-acetyldihydrodiketoeburicoate prepared by methods (*a*) and (*b*) were identical.

*Methyl* O-*Acetyltetrahydrodiketoeburicoate*.—Zinc dust (1 g.) was added gradually to a boiling solution (yellow) of methyl *O*-acetyldihydrodiketoeburicoate (0·18 g.) in acetic acid (10 ml.) during 5 min. and the colourless mixture then heated under reflux for 1 hr. Dilution of the filtered solution with much water gave *methyl* O-*acetyltetrahydrodiketoeburicoate* which separated from methanol in colourless lustrous plates (0·14 g.), m. p. 190—191°, $[\alpha]_D^{20}$ +51° (*c*, 1·35) (Found: C, 73·0; H, 9·9. $C_{34}H_{54}O_6$ requires C, 73·1; H, 9·7%).

*Dihydrodiketoeburiconic Acid*.—A solution of chromic anhydride (0·07 g.) in acetic acid (2 ml.) was added dropwise in 1 hr. to dihydrodiketoeburicoic acid (0·2 g.) dissolved in acetic acid (7 ml.) kept at 40°. The mixture was then kept at 60° for 1 hr., treated with a little methanol to decompose unchanged chromic acid, and diluted with water. Crystallised from dilute methanol, the solid gave *dihydrodiketoeburiconic acid* in yellow needles (0·15 g.), m. p. 248—249°, $[\alpha]_D^{19}$ +133° (*c*, 1·03) (Found: C, 75·0; H, 9·3. $C_{31}H_{46}O_5$ requires C, 74·7; H, 9·3%). Prepared with ethereal diazomethane, the *methyl* ester was purified by means of aluminium oxide, and then from hot aqueous methanol, being obtained as an amorphous powder, $[\alpha]_D^{21}$ +116° (*c*, 0·38) (Found: C, 74·7; H, 9·6. $C_{32}H_{48}O_5$ requires C, 75·0; H, 9·4%).

*Reduction of Eburiconic Acid*.—A mixture of this acid (Part XVI, *loc. cit.*) (3·8 g.), dioxan (40 ml.), aluminium *iso*propoxide (from 1·4 g. of aluminium), and *iso*propyl alcohol (30 ml.) was heated in a distillation apparatus on the steam-bath for 8 hr.; 15 ml. of distillate were collected. The residual solution was poured into an excess of dilute hydrochloric acid, the alcohol and dioxan were removed with steam, and the solid was dried and dissolved in boiling benzene (500 ml.). On being kept this solution deposited an amorphous product (2 g.) which was dried, digested with boiling benzene (50 ml.), and then crystallised from alcohol, giving eburicoic acid, m. p. and mixed m. p. 287—288°, $[\alpha]_D^{23}$ +51° (*c*, 0·11; 4-dm. tube) (Found: C, 78·9; H, 10·8. Calc. for $C_{31}H_{50}O_3$: C, 79·1; H, 10·7%), which gave the acetate, m. p. and mixed m. p. 251—253°, $[\alpha]_D^{21}$ +46° (*c*, 2·89) (Found: C, 77·3; H, 10·4. Calc. for $C_{33}H_{52}O_4$: C, 77·3; H, 10·2%).

Evaporation of the benzene liquor (500 ml.) left on separation of the crude eburicoic acid left a pale yellow gum (1·7 g.) which on acetylation with acetic anhydride (5 ml.) and pyridine (5 ml.) on the steam-bath for 3 hr. gave rise to epi-O-*acetyleburicoic acid* which was crystallised from alcohol and then methanol, forming needles, m. p. 260—261°, $[\alpha]_D^{21}$ +0·05° (*c*, 1·6) (Found: C, 76·9; H, 10·3%). Mixed with *O*-acetyleburicoic acid or with *O*-acetyldehydroeburicoic acid, it melted at about 230°; it did not depress the melting point of *epi*-*O*-acetyldehydroeburicoic acid. Deacetylation of this acetate gave epi*eburicoic acid*, forming needles, m. p. 257—259°, $[\alpha]_D^{21}$ +15° (*c*, 1·74), from alcohol (Found, in specimen sublimed in high vacuum: C, 78·9; H, 10·6%). This compound is considerably more soluble in chloroform, benzene, or alcohol than is eburicoic acid.

*Reduction of Dehydroeburiconic Acid*.—This acid (0·48 g.) was reduced with aluminium *iso*propoxide for 7 hr. and the crude product was dissolved in warm benzene (50 ml.). On being kept the cooled solution deposited a gelatinous solid which on crystallisation from alcohol gave

dehydroeburicoic acid, m. p. and mixed m. p. 286—288°, $[\alpha]_D^{24}$ +39° (*c*, 0·16; 4-dm. tube) (Found: C, 79·7; H, 10·5%); the acetate had m. p. and mixed m. p. 253—255°, $[\alpha]_D^{23}$ +62° (*c*, 0·98) (Found: C, 77·5; H, 10·0%). The benzene filtrate from the gelatinous solid was concentrated, and the residual liquor (25 ml.) kept for 2 days and, after the removal of a small amount of precipitate, evaporated. Treatment of the residue with warm acetic anhydride–pyridine gave epi-O-*acetyldehydroeburicoic acid* which separated from alcohol in needles, m. p. 262—263°, $[\alpha]_D^{23}$ +21° (*c*, 1·18), $\lambda_{max.}$ 235, 243, 252 mμ (log ε 4·17, 4·25, 4·07) (Found: C, 78·0; H, 10·0%). Mixed with *O*-acetyldehydroeburicoic acid or *O*-acetyleburicoic acid it melted at about 235°. Deacetylation of this acetate gave epi*dehydroeburicoic acid*, forming needles, m. p. 252—254°, $[\alpha]_D^{22}$ +22° (*c*, 0·2), from alcohol (Found, in specimen sublimed in high vacuum: C, 79·6; H, 10·5%).

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