## 196. *Furano-compounds. Part III. Euparin.*

By Bua Kamthong and Alexander Robertson.

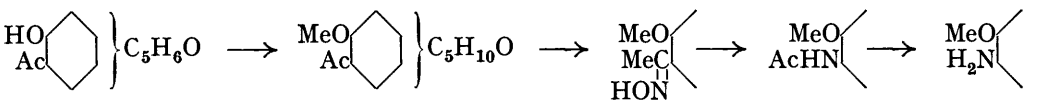
The yellow constituent, euparin, of *Eupatorium purpureum* has been shown to be a phenolic ketone $C_{13}H_{12}O_3$, forming derivatives with carbonyl reagents and a methyl ether and an acetate. Since they form tetrahydro-derivatives and give adducts with maleic anhydride, euparin and its methyl ether have two conjugate ethylenic linkages. The presence of a *C*-acetyl group, which is in the *o*-position to the hydroxyl group, is shown by the fact that *O*-methyltetrahydroeuparin oxime is transformed by Beckmann's method into an acetanilide giving the amine on hydrolysis which on acetylation re-forms the anilide. On oxidation with potassium permanganate the ether gave 2-hydroxy-4-methoxy-5-acetylbenzoic acid, and on ozonolysis 2-hydroxy-4-methoxy-5-acetylbenzaldehyde is formed. It is shown that euparin is a hydroxy-*C*-acetyl-coumarone or -chromen and possible formulæ are suggested.

The yellow crystalline principle, m. p. 117·2°, from the roots of *Eupatorium purpureum* (gravel root) appears to have been examined first by Trimble (*Amer. J. Pharm.*, **62**, 73) in 1890, who mentions that the compound had been described to him by Lloyd in a private communication between 1870 and 1875 and that the latter had named it euparin. To this substance Trimble assigned the formula $C_{12}H_{11}O_3$ and later Manger (*Amer. J. Pharm.*,

1894, **66**, 120) claimed to have confirmed this formula and by the action of ammonia on a liquid chloro-derivative to have obtained a compound $C_{24}H_{25}Cl_7O_6$. Further, the latter author, who was unable to prepare an acetyl derivative, noted the intense green ferric reaction given by the substance in alcohol and concluded from somewhat slender evidence that on treatment with nitric acid it gave rise to picric acid and on fusion with alkali furnished phloroglucinol.

In the course of an examination of the constituents of gravel root we have, by an improved method, isolated euparin, m. p. 118·5°, which appears to be identical with the material described by Trimble and by Manger (*loc. cit.*) but we have been unable to confirm the formula suggested by these authors. The analytical results given by euparin agree closely with the empirical formula $C_{13}H_{12}O_3$ and (less well) with $C_{17}H_{16}O_4$, but as the result of the examination of a large number of derivatives of euparin the latter possibility is entirely excluded. Under the usual conditions euparin, which is optically inactive and does not contain a carboxyl group, gave rise to a *monoacetate*, a *monomethyl* ether, an *oxime*, a *semicarbazone* and a 2 : 4-*dinitrophenylhydrazone*, thus affording clear proof that the molecule contains a hydroxyl and a carbonyl group. Further, in conjunction with the fact that the substance is sparingly soluble in dilute aqueous sodium hydroxide, the strong ferric reaction exhibited by euparin in alcohol indicates that the hydroxyl is phenolic and is in the *o*-position to the carbonyl group.

Hydrogenation of euparin, which is accompanied by disappearance of the colour, clearly established the presence of two ethylenic linkages in the molecule, yielding *tetrahydroeuparin* which, like euparin, formed an *oxime*, a 2 : 4-*dinitrophenylhydrazone*, an *acetate* and a *methyl* ether, identical with the product formed by the hydrogenation of *O*-methyleuparin. The resistance of the carbonyl group in euparin and *O*-methyleupa in to reduction with hydrogen and a palladium catalyst afforded strong evidence that this group was present as a ketonic residue and in the course of attempts to elucidate the nature of the latter it was found that euparin was unaffected with boiling 30% alcoholic sodium hydroxide and more drastic treatment with alkali did not give promising results. Ultimately, however, the compound was shown to contain a *C*-acetyl group by the following method :



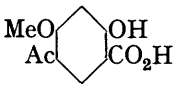

(I.) (II.) (III.) (IV.) (V.)

On treatment with thionyl chloride the *oxime* of *O*-methyltetrahydroeuparin underwent a Beckmann transformation and furnished an almost quantitative yield of an *amide* (IV), which on hydrolysis with alcoholic potassium hydroxide gave rise to the *aniline* (V) by loss of an acetyl residue. The nature of the amino-group in the latter product was clearly established by the fact that this substance formed a diazonium salt which coupled with β-naphthol to give a bright scarlet dye. On acetylation the amine (V) re-formed the Beckmann product (IV), thus affording conclusive proof of the presence of a *C*-acetyl residue in *O*-methyltetrahydroeuparin and hence euparin and *O*-methyltetrahydroeuparin may be represented by the structures (I) and (II) respectively.

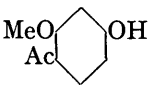
Oxidation of *O*-methyleuparin, dissolved in acetone, with aqueous potassium permanganate gave an acid $C_8H_6O_2(OMe)\cdot CO_2H$ in moderately good yield, which must be 2-*hydroxy*-4-*methoxy*-5-*acetylbenzoic acid* (VI) because on demethylation and methylation respectively it gave rise to 2 : 4-dihydroxy- and 2 : 4-dimethoxy-5-acetylbenzoic acid, identical with authentic specimens prepared according to Lindermann and Lindenbaum (*Ber.*, 1908, **41**, 1610), and on decarboxylation by the quinoline method to *iso*paeonol (VII). The conversion of (VI) into (VII) also serves to confirm the orientation of Lindermann and Lindenbaum's resacetophenone-carboxylic acid which was formed by application of the Friedel–Crafts reaction to β-resorcylic acid and therefore might have been 2 : 4-dihydroxy-3-acetylbenzoic acid (compare Baker, J., 1934, 1684).

In conjunction with the experiments on *O*-methyltetrahydroeuparin oxime these

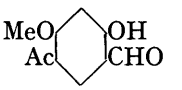
results clearly show that *O*-methyleuparin contains an *iso*paeonol residue and hence euparin itself is a derivative of resacetophenone. Further, the *C*-atom which appears as the carboxyl group in (VI) is part of a $C_5$ residue which carries two ethylenic linkages and forms an ether system with the oxygen appearing as the free phenolic hydroxyl group in (VI), thus accounting for the function of the third oxygen atom.



(VI.)

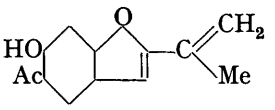


(VII.)

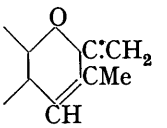


(VIII.)

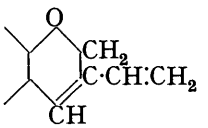
Ozonolysis of euparin methyl ether gave rise to formaldehyde and a phenolic *aldehyde* having an intense ferric reaction, which, in view of the production of (VI) by means of potassium permanganate, is considered to have the structure (VIII) and hence, on the assumption that the degradation by ozone proceeds in the normal manner (compare xanthoxyletin, J., 1936, 627), the remaining four carbon atoms of the $C_5$ residue are attached by an ethylenic linkage to the C-atom appearing as the formyl group in (VIII). The formation of formaldehyde by ozonolysis affords evidence that the second ethylenic linkage is present in the $C_5$ residue as a vinyl group and, further, the fact that both euparin and its methyl ether form crystalline adducts with maleic anhydride under the usual conditions indicates clearly that the ethylenic linkages present in the latter residue form a conjugate system.



(IX.)



(X.)



(XI.)

On the basis of these results it seems reasonably certain that the $C_5$ residue forms part of a heterocyclic ring system of the type (IX), (X) or (XI) where, it is of interest to note, in each case the $C_5$ unit has the isoprene skeleton; the remaining possible expressions for the $C_5$ residue appear to be definitely excluded by the analytical evidence and need not be dealt with at this stage. Of the formulæ (IX), (X) and (XI), (XI) appears to be the least likely on the basis of the formation of (VIII) from *O*-methyleuparin with ozone, but in view of the abnormal results obtaining with xanthoxyletin and xanthyletin (*loc. cit.*) this expression cannot be altogether excluded. By analogy with rotenone and the furocoumarins of the peucedanin group we prefer, of the remaining alternatives, formula (IX) for euparin.

## Experimental.

*Euparin.*—Finely powdered gravel root (30 lb., in batches of 10 lb.) was extracted (Soxhlet) with ether for 6 days and on evaporation of the solvent a green oil (698 g.) was obtained which after a long time deposited only traces of colourless crystalline material. Attempts to isolate euparin from this oil by means of solvents were unsuccessful and the following convenient and economical procedure was adopted: Light petroleum (b. p. 40—60°) (5 l.) was added to a solution of the green oil in twice its volume of ether (agitate) and 24 hours later the liquid was decanted from the resinous precipitate and evaporated. The residue was again treated with ether, followed by light petroleum, and an oil (A) (543 g.) was obtained which formed a clear solution with light petroleum; approximately 150 g. of a resin insoluble in light petroleum were thus separated. This material (A) (5 g.) was agitated with 10% aqueous sodium hydroxide (60 c.c.) for 5 minutes, the mixture extracted with ether, and the dried extracts evaporated; on being triturated with light petroleum (b. p. 40—60°), the residue yielded crude euparin as a yellow solid, m. p. 112—117°. By this method 543 g. of oil (A) gave 48·2 g. of euparin, 200·8 g. of a green oil obtained by acidification of the alkaline liquors, and 224 g. of a yellow oil resulting from the evaporation of the light petroleum washings of the euparin fraction. The last two fractions and the resinous precipitate are being retained for further investigation.

On being repeatedly crystallised from dilute alcohol and then from light petroleum (b. p. 80—100°), euparin formed squat, bright yellow prisms, m. p. 118·5°, which gave a green coloration with alcoholic ferric chloride and were readily soluble in ether, benzene or chloroform and very sparingly soluble in 8% aqueous sodium hydroxide [Found : C, 72·2; H, 5·5; *M* (Rast), 241, 254. Calc. for $C_{13}H_{12}O_3$ : C, 72·2; H, 5·6%; *M*, 216. Calc. for $C_{17}H_{16}O_4$ : C, 71·9; H, 5·6%; *M*, 284]. Euparin is volatile in steam and on being heated in a vacuum sublimes unchanged. Prepared by means of an alcoholic solution of 2 : 4-dinitrophenylhydrazine hydrochloride, the 2 : 4-*dinitrophenylhydrazone* formed dark brown prisms, m. p. 252°, from ethyl acetate (Found : N, 14·2. $C_{19}H_{16}O_6N_4$ requires N, 14·1%). The *oxime* separated from dilute alcohol in colourless prisms, m. p. 147—148° (Found : N, 6·2. $C_{13}H_{13}O_3N$ requires N, 6·1%), and the *semicarbazone* from much ethyl acetate in yellow prisms, m. p. 255° (Found : N, 15·4. $C_{14}H_{15}O_3N_3$ requires N, 15·4%).

Acetylation of euparin with excess of acetic anhydride and pyridine at room temperature for 3 days gave rise to the *acetate*, which formed colourless prisms, m. p. 80°, from light petroleum (b. p. 80—100°), having a negative ferric reaction (Found : C, 69·6; H, 5·5. $C_{15}H_{14}O_4$ requires C, 69·8; H, 5·4%).

Prepared by means of methyl iodide and potassium carbonate in boiling acetone in the course of 6 hours, O-*methyleuparin* formed colourless needles, m. p. 76—77°, from dilute alcohol, having a negative ferric reaction and readily soluble in the usual organic solvents [Found : C, 73·3; H, 6·0; OMe, 12·8. $C_{13}H_{11}O_2(OMe)$ requires C, 73·0; H, 6·1; OMe, 13·5%].

*Tetrahydroeuparin.*—Hydrogen (approx. 2 mols.) was absorbed at atmospheric pressure in the course of 3 hours by euparin (1 g.) dissolved in ethyl acetate (100 c.c.) containing a palladium–charcoal catalyst (from 0·2 g. of palladium chloride and 2 g. of charcoal); approximately half the hydrogen was absorbed in the course of 5 minutes. On isolation the *product* separated from light petroleum (b. p. 80—100°) in colourless rhombic prisms, m. p. 71°, readily soluble in alcohol, benzene or acetone and giving a brownish-red ferric reaction in alcohol (Found : C, 71·2; H, 7·4; *M*, 221, 227. $C_{13}H_{16}O_3$ requires C, 70·9; H, 7·3%; *M*, 220. $C_{13}H_{16}O_3$ requires C, 71·6; H, 6·4%; *M*, 220). Formed by the pyridine method, the *acetate* crystallised from light petroleum in colourless needles, m. p. 96—97°, having a negative ferric reaction (Found : C, 68·8; H, 6·9. $C_{15}H_{18}O_4$ requires C, 68·7; H, 6·9%). By the usual procedure tetrahydroeuparin gave rise to an *oxime*, forming colourless needles, m. p. 133°, from light petroleum (Found : N, 5·9. $C_{13}H_{17}O_3N$ requires N, 6·0%), and a 2 : 4-*dinitrophenylhydrazone*, scarlet prisms, m. p. 240—241°, from benzene (Found : N, 13·9. $C_{19}H_{20}O_6N_4$ requires N, 14·0%)

O-*Methyltetrahydroeuparin* was prepared from tetrahydroeuparin by the potassium carbonate–acetone method in the course of 6 hours and separated from light petroleum (b. p 80—100°) in colourless plates, m. p. 57°, having a negative ferric reaction [Found : C, 72·1; H, 7·7; OMe, 13·4. $C_{13}H_{15}O_2(OMe)$ requires C, 71·8; H, 7·7; OMe, 13·3%]. The same compound was obtained by hydrogenation of euparin methyl ether (0·5 g.), dissolved in ethyl acetate (50 c.c.), with hydrogen (approx. 2 mols. absorbed) at atmospheric pressure and a palladium–charcoal catalyst in the course of 2 hours and on purification had m. p. and mixed m. p. 57°.

The *oxime* of this compound formed colourless needles, m. p. 139°, from light petroleum (b. p. 60—80°) [Found : C, 67·5; H, 8·0; N, 5·8; OMe, 12·5. $C_{13}H_{16}O_2N(OMe)$ requires C, 67·5; H, 7·6; N, 5·6; OMe, 12·5%]. Purified thionyl chloride (0·9 c.c.) was added dropwise to a solution (agitate) of this oxime (1·5 g.) in absolute ether (100 c.c.) at — 5° in the course of 10 minutes. The colourless precipitate which first separated gradually became red and 15 minutes later the mixture was poured into ice-water (100 g.). After spontaneous evaporation of the ether the pale pink *amide* was collected; it crystallised from light petroleum (b. p. 80—100°) in colourless leaflets (0·9 g.), m. p. 133—134°, readily soluble in methyl or ethyl alcohol [Found : C, 67·4; H, 7·9; N, 5·4; OMe, 12·2. $C_{13}H_{16}O_2N(OMe)$ requires C, 67·5; H, 7·6; N, 5·6; OMe, 12·5%]. A second product could not be isolated from the residual aqueous liquors.

The amide (0·6 g.) was boiled with 20% alcoholic potassium hydroxide (10 c.c.) for 4 hours, and the crystalline product precipitated with water. A solution of this material in dilute hydrochloric acid (3 c.c.) was filtered to remove traces of insoluble impurities and, on being basified with aqueous sodium hydroxide, gave the *amine*, which was purified by crystallisation from a small volume of light petroleum and then from aqueous methyl alcohol and finally by sublimation in a high vacuum, being obtained in colourless plates, m. p. 72° (Found : N, 6·8.

$C_{12}H_{17}O_2N$ requires N, 6·8%). Acetylation of the base (0·4 g.) with acetic anhydride (0·5 g.) during 5 minutes re-formed the amide (0·4 g.), m. p. and mixed m. p. 133—134°, after purification from light petroleum (Found: C, 67·4; H, 7·7; N, 5·7; OMe, 12·6%).

*Oxidation of* O-*Methyleuparin with Potassium Permanganate.*—4% Aqueous potassium permanganate (110 c.c.) was added to a well-agitated solution of the ether (1 g.) in acetone (30 c.c.) in the course of 4 hours and after the addition of dilute sulphuric acid (5 c.c.) the mixture was cleared with sulphur dioxide, warmed on the water-bath for 20 minutes, cooled, and extracted ten times with ether. The light brown solid (0·43 g.) left on evaporation of the combined dried ethereal extracts was dissolved in aqueous sodium bicarbonate, traces of insoluble material removed by means of ether, the residual solution acidified with hydrochloric acid, and the acidic product isolated with ether and crystallised from dilute alcohol and then from much warm water, giving 2-*hydroxy*-4-*methoxy*-5-*acetylbenzoic acid* in colourless needles, m. p. 215—217° (decomp.) after darkening at 211°, sparingly soluble in benzene, insoluble in light petroleum, and having a red-brown ferric reaction in alcohol [Found: C, 56·9; H, 4·8; OMe, 14·3; *M* (by titration), 216·9. $C_9H_7O_4(OMe)$ requires C, 57·1; H, 4·8; OMe, 14·8%; *M*, 210]. This acid (0·5 g.) was esterified by means of excess of ethereal diazomethane and the product, which was insoluble in aqueous sodium bicarbonate and gave a positive ferric reaction, was methylated with excess of methyl iodide and potassium carbonate (1 g.) in boiling acetone (20 c.c.) during 8 hours. On isolation and hydrolysis the resulting ether gave rise to 2 : 4-dimethoxy-5-acetylbenzoic acid (0·4 g.), which separated from warm alcohol in colourless needles, m. p. 233—234°, having a negative ferric reaction and identical in every way with an authentic specimen (Lindermann and Lindenbaum, *loc. cit.*, who give m. p. 231—233° but do not appear to have analysed their specimen) [Found: C, 58·9; H, 5·5; OMe, 26·6. Calc. for $C_9H_6O_3(OMe)_2$: C, 58·9; H, 5·4; OMe, 27·7%].

The foregoing oxidation product (0·5 g.) was decarboxylated by being heated (oil-bath at 200—220°) with quinoline and copper-bronze for 20 minutes and on isolation the resulting ketone was triturated with light petroleum and then crystallised from water, forming colourless needles, m. p. 135—137°, identical with an authentic specimen of 2-*O*-methylresacetophenone and yielding a 2 : 4-*dinitrophenylhydrazone* which separated from alcohol in dark red prisms, m. p. 216—217° (Found: N, 16·1. $C_{15}H_{14}O_6N_4$ requires N, 16·2%). Authentic material was prepared by the method of Hoesch (*Ber.*, 1915, **48**, 1126) and also by the following procedure: 2-Hydroxy-4-benzyloxyacetophenone, m. p. 105—106° (J., 1937, 1535), was methylated with excess of methyl iodide and potassium carbonate in boiling acetone during 9 hours and the resulting 2-*methoxy*-4-*benzyloxyacetophenone* (1 g.), which separated from alcohol in prisms, m. p. 69° [Found: OMe, 11·9. $C_{15}H_{13}O_2(OMe)$ requires OMe, 12·1%], was debenzylated by being heated on the water-bath for 1 hour and then under reflux for 5 minutes with a mixture of acetic acid (25 c.c.) and concentrated hydrochloric acid (15 c.c.). After isolation with ether and purification by means of 2% aqueous sodium hydroxide and then by crystallisation from water 2-*O*-methylresacetophenone had m. p. 136—137°.

The acid (0·3 g.) obtained by oxidation of euparin methyl ether with potassium permanganate was boiled (oil-bath) for 2 minutes with hydriodic acid (3 g., *d* 1·9) and the product (0·2 g.) which separated was collected, washed, and dissolved in aqueous sodium bicarbonate. After having been treated with charcoal and filtered, this solution on acidification yielded the 2 : 4-dihydroxy-5-acetylbenzoic acid, forming colourless prisms, m. p. 256° (decomp.), from alcohol, identical with an authentic specimen (Lindermann and Lindenbaum, *loc. cit.*).

*Ozonolysis of* O-*Methyleuparin.*—A slow stream of ozone and oxygen was led into a solution of the ether (1 g.) in chloroform (50 c.c.) during 1½ hours, the solvent was removed in a vacuum, and the residue was digested with water (50 c.c.) at room temperature for 15 hours and then on the water-bath for 20 minutes. On cooling, the solution slowly deposited 2-*hydroxy*-4-*methoxy*-5-*acetylbenzaldehyde*, which formed colourless needles (0·35 g.), m. p. 117—118°, from warm water (charcoal), having a brown ferric reaction in alcohol [Found: C, 61·7; H, 5·5; OMe, 15·7. $C_9H_7O_3(OMe)$ requires C, 61·9; H, 5·2; OMe, 16·0%].

The combined aqueous filtrate and washings from the crude aldehyde were distilled and on treatment with excess of 2 : 4-dinitrophenylhydrazine hydrochloride in hydrochloric acid the distillate gave a precipitate of the 2 : 4-dinitrophenylhydrazone of formaldehyde, which on crystallisation from alcohol had m. p. 163—164° (Found: N, 26·9. Calc. for $C_7H_6O_4N_4$: N, 26·7%).

*Condensation of Euparin and* O-*Methyleuparin with Maleic Anhydride.*—A solution of euparin (0·5 g.) and maleic anhydride (0·25 g.) in warm benzene (25 c.c.) was refluxed in the steam-bath for 14 hours and the *product*, which separated from the cooled mixture, was collected and

crystallised from alcohol, forming colourless needles (0·5 g.), m. p. 244—245° (Found : C, 64·7; H, 4·6. $C_{17}H_{14}O_6$ requires C, 65·0; H, 4·5%).

When a solution of *O*-methyleuparin (1 g.) and maleic anhydride (1 g.) in benzene or toluene (50 c.c.) was refluxed for 12 hours, the *adduct* separated in colourless plates, m. p. 212—213° after purification from alcohol [Found : C, 65·6; H, 5·0; OMe, 9·1. $C_{17}H_{13}O_5(OMe)$ requires C, 65·9; H, 4·9; OMe, 9·5%].

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