725. *The Oxygen Groups of Cassaic Acid.*

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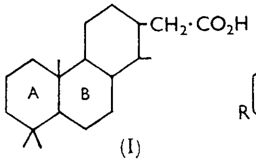
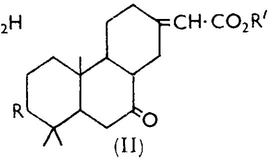
Dehydrogenation suggests the correctness of formula (II; $R = OH$, $R' = H$) for cassaic acid.

The carbon skeleton of cassaic acid [1,2] was confirmed by preparation of cassanic acid (I) from vouacapenic acid.[3] Cassaic acid has a double bond α to the carboxylic acid, and a keto- and a hydroxyl group, the last two being distributed between rings A and B of the phenanthrene nucleus. Formula (II; $R = OH$, $R' = H$) was put forward as a working hypothesis.[2,4] Its correctness is suggested by our dehydrogenation experiments.

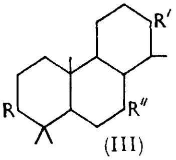
The position of the hydroxyl group has been shown as follows. Ozonolysis of methyl cassate acetate (II; $R = OAc$, $R' = Me$) furnished a diketone (III; $R = OAc$, $R' = R'' = O$) which on Wolff–Kishner reduction yielded perhydro-2-hydroxy-1,1,8,12-tetramethylphenanthrene (III; $R = OH$, $R' = R'' = H$), concomitant hydrolysis of the acetoxy-group taking place. Chromic anhydride–pyridine then smoothly gave the 2-oxo-compound and the position of this oxygen (the original hydroxyl group) was marked by means of the Grignard reagent: dehydrogenation afforded 1,2,8-trimethylphenanthrene.



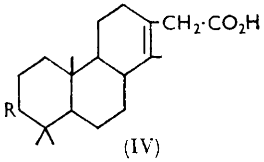
(I)



(II)



(III)



(IV)

The ketone group of cassaic acid which must therefore be at position 9 or 10 is rather inert since it resisted Clemmensen reduction. Dioxocassenic acid (II; $R = O$, $R' = H$) was therefore reduced under the same conditions, giving 9-oxocassenic acid (II; $R = R' = H$), in which a ketone group still survived, this interpretation being supported by the Zimmermann test: Barton and de Mayo [5] showed that this test, characteristic of steroidal 3-ketones, is given also by triterpenes with a ketone group in the same position (not by an 11-, 12-, 16-, or 19-ketone of the β-amyrin series or 7- or 11-ketone of the lanostane group). In the cassaic acid series only a 2- * or 7-ketone gave a colour with the Zimmermann reagent (see Experimental section) and since the acid (II; $R = R' = H$) obtained from the Clemmensen reduction above gave a negative Zimmermann test, we felt reasonably certain of the absence of a 2-ketone.† Attempts to synthesise the isomeric 2-oxocassenic acid were unsuccessful. Thus, although cassaic acid was inert to Clemmensen reduction, the Wolff–Kishner procedure gave a hydroxy-acid which showed no absorption above 200 mμ and was presumably the allo-compound. (IV; $R = OH$). Oxidation with chromic anhydride yielded the corresponding 2-oxoallocassenic acid (IV; $R = O$).

Ozonolysis of the acid (II; $R = R' = H$) yielded a dioxo-compound (III; $R = H$, $R' = R'' = O$), which on treatment with methyl-lithium followed by selenium dehydrogenation afforded 1,7,8,9-tetramethylphenanthrene. Formula (II; $R = R' = H$) for cassaic acid, is thus confirmed.‡

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* Steroidal $C_{(3)} \equiv C_{(2)}$ of the perhydrophenanthrene structure.

† Cf. the reduction of 3,7,12-trioxocholanic acid to 7,12-dioxocholanic acid.[6]

‡ During preparation of this manuscript a preliminary note [7] appeared in which much of the stereochemistry and the position of the oxygen atom in ring B have been elegantly demonstrated.

[1] Dalma, *Helv. Chim. Acta*, 1939, **22**, 1497; Ruzicka and Dalma, *ibid.*, p. 1516; 1940, **23**, 753; Ruzicka, Dalma, and Scott, *ibid.*, 1941, **24**, 179E; Ruzicka, Engel, Ronco, and Berse, *ibid.*, 1945, **28**, 1038.

[2] Humber and Taylor, *J.*, 1955, 1044.

[3] King, King, and Uprichard, *J.*, 1958, 3428.

[4] Tondeur, Ph.D. Thesis, Eidgen. Techn. Hochschule, Zurich, 1950.

[5] Barton and de Mayo, *J.*, 1954, 887.

[6] Borsche, *Ber.*, 1919, **52**, 1363.

[7] Turner, Herzog, Morin, and Riebel, *Tetrahedron Letters*, 1959, No. 2, p. 7.

During our work Professor W. J. Gensler of Boston University kept us informed of his similar findings, partly published already (ref. 9). We are very grateful for such information before publication and for a specimen of 1,7,8,9-tetramethylphenanthrene which he synthesised for the first time.

Ozonolysis of dioxocassenic acid (II; R = O, R′ = H) to give the triketone (III; R = R″ = R‴ = O) has been reported by Engel.[8] We find that the same triketone is also obtained from cassaic acid itself, the 2-hydroxyl group being oxidised. By contrast, a 9-hydroxyl group is inert and the product of ozonolysis of cassaidic acid contains a hydroxyl group: since this product yields a purple colour with the Zimmermann reagent, it is probably the hydroxy-diketone (III; R = R′ = O, R″ = OH). In addition to the differing reactivity of the 2- and the 9-oxygen atom with amalgamated zinc or ozone, it has already been reported [9] that 2- but not 9-ketones react with the Grignard reagent.

EXPERIMENTAL

The bark of *Erythrophleum guineense* (G. Don) came partly from Sierra Leone, and partly from the Belgian Congo. We thank the Colonial Products Council and l'Institut National pour l'Étude Agronomique du Congo Belge (INEAC) for their generous help in supplying botanical material. The bark was extracted essentially as described by Ruzicka, Dalma, and Scott [10] for *Erythrophleum couminga.*

Cassaine Hydrogen Sulphate.—The crude alkaloidal fraction was dissolved in dry ether and, with stirring, 3% sulphuric acid in ether was added at 20° until no further precipitate formed. The mixture was kept at 0° for several hours and the crude precipitate then collected. The yield of cassaine hydrogen sulphate varied in the limits 4—14%. Crystallisation of crude material from methanol gave colourless plates, m. p. 289—290° (decomp.), $[\alpha]_D^{24}$ −96° (in ethanol, *c* 1).

Cassaic Acid.—Cassaine hydrogen sulphate (6 g.) was heated at 100° with 2N-hydrochloric acid (220 ml.). After 2 hr. the oily material which separated initially had become granular (yield, 3·15 g.). Crystallisation from ethanol–ether gave prisms, m. p. 218—220°, $[\alpha]_D^{20}$ −124° (in ethanol, *c* 1·2) (Dalma [1] gives m. p. 203°, $[\alpha]_D^{20}$ −126°), $\nu_{max.}$ (in Nujol mull) 3500 (OH), 2500 (OH), 1710 (C=O), 1678 (CO_2H), 1635 (C=C), 1200 (CO_2H) cm.$^{-1}$. Cassaic acid gave no colour with the Zimmermann reagent.

Cassaic acid formed a p-*phenylphenacyl ester*, m. p. 195—197° (from aqueous ethanol), $[\alpha]_D^{24}$ −108° (in chloroform, *c* 1) (Found: C, 77·6; H, 7·7. $C_{34}H_{40}O_5$ requires C, 77·3; H, 7·6%). Methyl cassate formed an *oxime*, m. p. 108—110° (from ether–light petroleum) (Found: N, 3·7. $C_{21}H_{33}O_4N$ requires N, 3·85°).

Methyl cassate formed a 2,4-*dinitrophenylhydrazone*, orange needles (from ethyl acetate–ethanol), m. p. 145—147°, $\lambda_{max.}$ (main band in ethanol) 361 mμ (log ε 4·3) (Found: C, 61·2; H, 6·7; N, 10·7. $C_{27}H_{36}O_7N_4$ requires C, 61·3; H, 6·8; N, 10·6%).

2-*Acetoxyperhydro*-1,1,8,12-*tetramethyl*-7,9-*dioxophenanthrene* (III) (R = OAc, R′ = R″ = O).—Methyl cassate (695 mg.) in dry chloroform (85 ml.) was treated with 5—6% ozonised oxygen for 90 min. at −20° to −40°. The resulting blue solution was allowed to attain room temperature in 3 hr. and the solvent was then removed. The gummy ozonide (760 mg.) in glacial acetic acid (20 ml.) was treated with powdered zinc (2 g.), shaken for 15 min., then intermittently for 2 hr. Unchanged metal was filtered off and the solvent was removed, being replaced by dilute hydrochloric acid (200 ml.). An ether extract yielded a neutral residue (570 mg.). Crystallisation from ether gave the acetoxy-diketone in plates, m. p. 162·5—164° (Found: C, 71·7; H, 8·8. $C_{20}H_{30}O_4$ requires C, 71·9; H, 9·0%), $\nu_{max.}$ (in bromoform) 1720 (C=O) and 1240 cm.$^{-1}$ (OAc).

The acetoxy-diketone (15 mg.) with N-alcoholic potassium hydroxide to 100° for 2 hr. gave the corresponding hydroxy-diketone, m. p. 173—175° (from ether–light petroleum), $[\alpha]_D^{20}$ −68° (in ethanol, *c* 0·8). Neither the acetoxy- nor the hydroxy-compound gave a colour with the Zimmermann reagent.

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[8] Engel, *Helv. Chim. Acta*, 1959, **42**, 131.
[9] Gensler and Sherman, *Chem. and Ind.*, 1959, 223.
[10] Ruzicka, Dalma, and Scott, *Helv. Chim. Acta*, 1941, **24**, 63.

Perhydro-1,1,8,12-*tetramethyl*-2,7,9-*trioxophenanthrene* (III; R = R′ = R″ = O).—Cassaic acid (200 mg.) in glacial acetic acid (20 ml.) was ozonised as above. The ozonide was decomposed by warm water (~15 ml.), and on working up in the usual fashion a neutral fraction resulted (75 mg.). Crystallisation from ether–light petroleum gave the triketone in needles, m. p. 182—183°, $[\alpha]_D$ $-75°$ (in ethanol, *c* 0·8) (Engel[8] gives m. p. 198·5—200°), $[\alpha]_D^{21}$ $-61°$) (Found: C, 73·9; H, 8·6. Calc. for $C_{18}H_{26}O_3$: C, 74·5; H, 9·0%).

Passing this material in ether through an alumina column afforded higher-melting material, 197—198·5°, $\lambda_{max.}$ (in ethanol) 289 mμ (log ε 1·81), $\nu_{max.}$ (in bromoform) 1706 cm.$^{-1}$ (C=O) (no OH bands). This change in m. p. probably indicates a change in conformation of the 8-methyl group.

The same triketone was (m. p. and mixed m. p. 182—184°) obtained on ozonolysis of methyl dioxocassenate. It gave a purple colour with the Zimmermann reagent.

Sodium carbonate extracts from purification of the ozonolysis product from cassaic acid were acidified, evaporated to 10—15 ml., then extracted with ether in a liquid–liquid extractor. The ethereal extract was washed with 20% aqueous ammonia and to this solution of ammonium salts was added a concentrated calcium chloride solution till no further precipitate of calcium oxalate was obtained. The isolated calcium oxalate (51 mg.) was then decomposed with dilute sulphuric acid, and the precipitated calcium sulphate centrifuged off. The filtrate was titrated with 0·2N-sodium hydroxide, made just acid with hydrobromic acid, and refluxed with 4-nitrobenzyl bromide (26 mg.) in ethanol (5 ml.) for 1½ hr. The ester which separated crystallised from ethyl alcohol, had m. p. 204° (decomp.) alone or mixed with di-4-nitrobenzyl oxalate.

Perhydro-2-*hydroxy*-1,1,8,12-*tetramethylphenanthrene* (III; R = OH, R′ = R″ = H).—The acetoxy-diketone (200 mg.) was added to redistilled diethylene glycol (10 ml.) in which sodium (100 mg.) had dissolved. Sufficient anhydrous hydrazine was distilled into this to ensure that the reflux temperature was 180°; refluxing was continued at this temperature for 24 hr., then sufficient hydrazine was distilled out to raise the temperature to 210°, a further 24 hours' refluxing being allowed. The cooled mixture was poured into 0·5N-hydrochloric acid and extracted with ether. From this a neutral residue (120 mg.) resulted. This crystallised from ether–light petroleum to give the *product* as prisms, m. p. 147—148°, $[\alpha]_D^{21}$ $+28°$ (in ethanol, *c* 1) (Found: C, 81·6; H, 11·9; O, 6·0. $C_{18}H_{32}O$ requires C, 81·9; H, 12·1; O, 6·1%), $\nu_{max.}$ (KBr disc) 3416 cm.$^{-1}$ (OH) (C=O bands were absent), giving no colour with the Zimmermann reagent.

This product (230 mg.) with chromic anhydride gave (75 mg.) in pyridine (0·75 ml.) a gummy ketone ($\nu_{max.}$ 1703 cm.$^{-1}$), which afforded a positive Zimmermann test.

The crude ketone (210 mg.) in anhydrous ether (50 ml.) was added dropwise to methylmagnesium iodide (from 100 mg. of Mg). After 1 hour's refluxing the mixture was worked up in the usual manner to yield a gummy alcohol (217 mg.). This was heated with selenium (180 mg.) at 340° for 24 hr.; the mixture was extracted with ether, and the extract filtered through a short column of alumina. The eluate, with picric acid, yielded a picrate (0·088 g.), which on crystallisation from alcohol had m. p. 162—163° alone or mixed with 1,2,8-trimethylphenanthrene picrate. By passing the picrate through a column of alumina (Grade I) and collecting crystalline fractions, 44 mg. of hydrocarbon, m. p. 136—139°, were obtained. Two crystallisations from ethanol followed by sublimation at 140°/10^{-5} mm. gave plates, m. p. and mixed m. p. 145—146°. The two specimens had $\lambda_{max.}$ (in ethanol) 211, 224, 262, 282, 293, 307, 339 mμ (log ε 4·60, 4·39, 4·80, 4·13, 4·13, 4·23, 2·63).

2,9-*Dioxocassenic Acid* (II; R = O, R′ = H).—Cassaic acid (30 mg.) was oxidised as described by Ruzicka, Dalma, and Scott,[10] to yield 21 mg. of diketo-acid, m. p. 246—248°, $[\alpha]_D^{21}$ $-159°$ (in ethanol, *c* 0·8) (Ruzicka *et al.*,[10] m. p. 249°, $[\alpha]_D^{20}$ $-152°$), $\nu_{max.}$ (KBr disc) 1700 (C=O) and 1647 cm.$^{-1}$ (double bond α to carbonyl group) (Found: C, 72·3; H, 8·6. Calc. for $C_{20}H_{28}O_4$: C, 72·3; H, 8·4%). This compound gave a purple colour with the Zimmermann reagent.

9-*Oxocassenic Acid* (II; R = R′ = H).—2,9-Dioxocassenic acid (500 mg.) in acetic acid (40 ml.) was refluxed with amalgamated zinc (6 g.) concentrated hydrochloric acid (12 ml.) and water (8 ml.) for 8 hr. After working up in the usual manner an acidic solid was obtained (410 mg.; m. p. 180—185°). This was chromatographed in benzene–ether (1 : 2) on M.F.C. silica gel (20 g.) (24 × 1 cm.). The chromatogram was developed with benzene–ether (1 : 2) (200 ml.). Crystalline fractions (400 mg.) were obtained with benzene–ether (1 : 4) and, recrystallised from ether–light petroleum, had m. p. 188—189·5°. 9-*Oxocassenic acid* gave a precipitate with 2,4-dinitrophenylhydrazine, but failed to react in the Zimmermann test;

it had $[\alpha]_D^{20}$ −70° (in ethanol, *c* 0·6), $\lambda_{max.}$ (in ethanol) 220 mμ (log ε 4·2), $\nu_{max.}$ (in chloroform) 1710 (C=O) and 1650 cm.$^{-1}$ (C=C) (Found: C, 75·1; H, 9·8. $C_{20}H_{30}O_3$ requires C, 75·5; H, 9·5%).

Perhydro-1,1,8,12-*tetramethyl*-7,9-*dioxophenanthrene* (III; R = H, R′ = R″ = O).—9-Oxocassenic acid (370 mg.) in dry chloroform (90 ml.) was ozonised at −20° to −30° for 65 min., then left to warm to room temperature for 3 hr. Solvent was removed. The resulting ozonide in glacial acetic acid (20 ml.) was decomposed by shaking for 3 hr. with powdered zinc (3 g.) in water (20 ml.), a neutral gum (230 mg.) being obtained. This could not be satisfactorily purified and after subliming twice at 110/10^{-5} mm. was used in the next stage. This gum gave a purple colour with the Zimmermann reagent.

The crude diketone (210 mg.) in dry ether (80 ml.) was treated with ethereal methyl-lithium (from lithium, 1 g., and methyl iodide, 12 g.). The resulting gummy alcohol was heated with selenium (230 mg.) at 340° for 48 hr., then extracted with ether. The extract was washed with sodium hydroxide solution, and afforded a neutral gum (44 mg.). After filtering in ether through alumina this was twice sublimed at 110°/10^{-5} mm., then crystallised twice from methanol, to yield plates (2 mg.), m. p. 108—110° alone or mixed with 1,7,8,9-tetramethylphenanthrene. The alcoholic mother-liquors from crystallisations were converted into the trinitrobenzene derivative, m. p. and mixed m. p. 157—159°, of 1,7,8,9-tetramethylphenanthrene. The ultraviolet absorption of the two specimens were identical [$\lambda_{max.}$ (in ethanol) 217·5, 230, 265, 286, 299, 311, 342, 359 mμ (log ε 4·57, 4·49, 4·75, 4·08, 4·06, 4·08, 2·92, 2·82)].

2-*Hydroxyallocassenic Acid.*—Cassaic acid (200 mg.) was reduced by the Wolff–Kishner method as modified by Barton, Ives, and Thomas.[11] There was thus obtained the above *compound*, m. p. 224—226° (from methanol), $[\alpha]_D^{20}$ −88° (in ethanol, *c* 1·0) (no absorption above 210 mμ), $\nu_{max.}$ (in Nujol mull) 3490 (OH) and 1708 cm.$^{-1}$ (C=O) (Found: C, 75·0; H, 10·2. $C_{20}H_{32}O_3$ requires C, 75·1; H, 10·0%). It gave no colour with the Zimmermann reagent.

When oxidised with chromic anhydride in the usual manner 2-*oxoallocassenic acid* was formed, crystallising from aqueous acetone in needles, m. p. 153—155°, $[\alpha]_D^{20}$ −92° (in ethanol, *c* 0·37), $\nu_{max.}$ (KBr disc) 1733 (CO_2H) and 1703 cm.$^{-1}$ (C=O) (Found: C, 74·9; H, 9·85. $C_{20}H_{30}O_3$ requires C, 75·5; H, 9·5%). The compound showed no absorption characteristic of an αβ-unsaturated carboxylic acid in the 210—230 mμ region and gave a purple colour with the Zimmermann reagent.

Cassaidic Acid.—Sodium borohydride (100 mg.) in water (5 ml.) containing 1 drop of 2N-sodium hydroxide was added dropwise at 35° to cassaic acid (200 mg.) in methanol (40 ml.). After 1 hr. solvent was removed and the residue cooled to 0°, then acidified with dilute hydrochloric acid. The resulting precipitate (178 mg.), recrystallised from ethyl acetate, had m. p. 275° (decomp.), $[\alpha]_D^{20}$ −102° (in ethanol, *c* 1·0) (lit., m. p. 275—277°), $[\alpha]_D^{20}$ −100°), $\nu_{max.}$ (in Nujol mull) 3500 (OH), 1692 (CO_2H), 1645 cm.$^{-1}$ (C=C). This acid formed a p-*phenylphenacyl ester*, plates (from ether–light petroleum), m. p. 113—116°, $[\alpha]_D^{22}$ −97·7° (in chloroform, *c* 1·0) (Found: C, 77·1; H, 7·7. $C_{34}H_{40}O_5$ requires C, 77·0; H, 8·0%). Cassaidic acid gave no colour with the Zimmermann reagent. Engel[12] showed that if dioxocassenic acid is treated with one mol. proportion of sodium borohydride only the 2-ketone group is reduced and cassaic acid is formed.

Ozonolysis of Methyl Cassaidate.—Methyl cassaidate (12 mg.) was ozonised in dry chloroform (12 ml.), yielding a substance which, crystallised from carbon tetrachloride, had m. p. 133—136°, $[\alpha]_D^{20}$ −58° (in chloroform, *c* 0·3), $\nu_{max.}$ 3500 (OH) and 1710 cm.$^{-1}$ (C=O). The compound gave a purple colour with the Zimmermann reagent.

We thank Dr. J. E. Page of Glaxo Laboratories Ltd. for determining the infrared spectrum of several compounds (on a Perkin–Elmer model 21 spectrophotometer), also the Department of Scientific and Industrial Research for a special research grant towards the purchase of an " Infracord " spectrophotometer. We are grateful to Dr. J. F. McGhie for a specimen of 1,2,8-trimethylphenanthrene and its picrate.

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[*Received, May 28th,* 1959.]

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[11] Barton, Ives, and Thomas, *J.*, 1955, 2056.

[12] Engel, *Helv. Chim. Acta*, 1959, **42**, 1127.