

Limonoids from *Khaya madagascariensis* Jumelle et Perrier

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The timber of *Khaya madagascariensis* contains the two new limonoids, 11 β -acetoxykhivorin and methyl 3 β -acetoxy-2-hydroxy-1-oxomeliacate. The seed yielded the known compounds fassinolide and 3-deacetylkhivorin.

Khaya madagascariensis Jumelle et Perrier is a rare mahogany, confined to the Comoro islands and a restricted area in N.W. Madagascar. We have examined the timber, root, bark, root bark, and seed of a specimen collected near Majunga, in Madagascar; the timber and root gave similar results. Petroleum extraction yielded a substance which crystallised from methanol-methylene chloride as large prisms, m.p. 165° (recrystallising and remelting at 218° and 268°). Chromatography of the

mother liquor gave a second compound, m.p. 240°, $[\alpha]_D^{20} = -64^\circ$. No limonoids were isolated from the bark or root bark. The seed gave the known limonoids methyl 3 β -acetoxy-1-oxomeliac-8,14-enate (I) (fassinolide^{1,2} nomenclature *cf.*³) and 3-deacetylkhivorin (IIa).⁴ The compound m.p. 165°/268° gave a molecular ion at 644, corresponding to an acetoxy-khivorin. Combustion analysis gave a poor result, since the crystals retain both methanol and methylene chloride. The n.m.r. spectrum

¹ R. Zelnik and C. Rosito, *Tetrahedron Letters*, 1966, 6441.

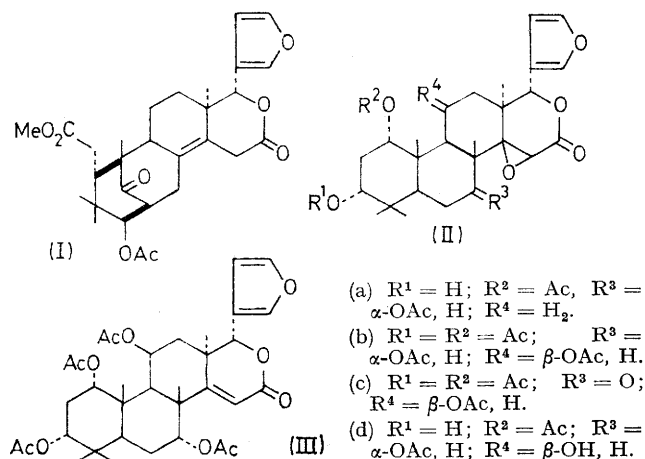
² E. K. Adesogan and D. A. H. Taylor, *Chem. Comm.*, 1967, 225.

³ E. K. Adesogan and D. A. H. Taylor, *J. Chem. Soc. (C)*, 1968, 1974.

⁴ E. K. Adesogan, J. W. Powell, and D. A. H. Taylor, *J. Chem. Soc. (C)*, 1967, 554.

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after evaporation of the sample with deuteriochloroform to remove other solvents, was largely similar to that of khivorin. The differences were shifts in the positions of the tertiary methyl group resonances, the presence of four acetates instead of three, and the presence of a



new 1H signal at δ 5.10 p.p.m. (quintet, J 4.5 c./sec.) and a new 1H doublet at δ 2.94 p.p.m. (J 4 c./sec.). Double-resonance experiments showed that these were coupled, as the quintet collapsed to a quartet on irradiation at δ 2.94 p.p.m. The quintet was also coupled (J 5 c./sec.) to a pair of doublets at δ 1.45 p.p.m.. On irradiation at δ 5.10 p.p.m., these collapsed to a doublet (J 15 c./sec.), which we think is half of an A/B pair of which the other half is concealed by the acetate bands. There are also three signals at δ 4.56 p.p.m. [J^* ($J_{AX} + J_{BX}$ in an ABX system) 5], δ 4.65 (J^* 6), and 4.74 p.p.m. (J^* 5 c./sec.); these correspond in chemical shift and coupling to the signals for 1-H, 3-H, and 7-H in khivorin. We consider, therefore, that the compound is an acetoxymeliacolide. To give the observed coupling, the fourth acetate group could only be at C-11, in which case the doublet at δ 2.94 p.p.m. represents 9-H and the group at δ 1.45 p.p.m. one of the protons at 12-H. Recently we have assigned the methyl group resonances in a series of limonoids,⁵ and have been able to calculate increments for the various substituents in the molecule. In this way, we find that the 11 β -acetoxymeliacolide shifts the 8- and 10-methyl group resonances downfield by 11 and 13 c./sec. respectively, at 60 Mc./sec., compared to the positions in gedunin. The corresponding shifts from khivorin in the present compound are 12 and 15 c./sec., in good agreement. Therefore, we propose that our compound is 11 β -acetoxymeliacolide (IIb). An 11 α -acetoxymeliacolide, as in anthothecol,⁷ would not be expected to produce a similar shift of the methyl groups. A preliminary account of this work has been given.⁸

Treatment of 11 β -acetoxymeliacolide with chromous

chloride gave a deoxy-compound (III). In the n.m.r. spectrum of this compound, the signal due to 11-H is concealed beneath those due to 17-H and 7-H, but the 9-H resonance is still visible at δ 2.73 p.p.m. (J 5.5 c./sec.). Mild alkaline hydrolysis of 11 β -acetoxymeliacolide gave a gum, which was chromatographed to give an amorphous mono-deacetyl and a crystalline di-deacetyl compound, together with an unresolved mixture. Oxidation of the mono-deacetyl compound gave a crystalline ketone. This was recognised as a 7-ketone by the characteristic downfield shift of the 15-H signal to δ 3.77 p.p.m.;⁹ it was, therefore, 14 β ,15 β -epoxy-7-oxo-1 α ,3 α ,11 β -triacetoxymeliacolide (IIc) (nomenclature *cf.* ref. 5). The increments for the 8- and 10-methyl resonances in this compound, compared to the corresponding khivorin derivative, are 8, 14 c./sec.; in reasonably good agreement with the previous values. The 100 Mc./sec. n.m.r. spectrum showed three downfield 1H signals at δ 4.8 (J^* 6), 4.9 (J^* 6 c./sec.), and 5.24 p.p.m. (pair of broad singlets $w_{1/2} = 3$ c./sec., J 6 c./sec.). Irradiation at δ 5.24 p.p.m. sharpened a broad singlet at δ 2.87 p.p.m., which must represent 9-H. A 2H singlet also appeared at δ 1.64 p.p.m., representing the two protons at C-12. In reverse, irradiation at δ 1.64 p.p.m. reduced the 11-H signal to a singlet. Irradiation of 1-H or 3-H showed the C-2 protons at δ 2.22 p.p.m. (broad singlet); irradiation at δ 2.22 p.p.m. reduced both 1-H and 3-H to sharp singlets. The coupling constants of 11-H show that the conformation of ring c has changed on oxidation at C-7. The coupling in 11 β -acetoxymeliacolide is the same as that in 11 β -acetoxymeliacolide, which Overton and his co-workers⁶ have ascribed to a twist-boat conformation of ring c, in which C-11 rotates so as to move the acetate away from 8- and 10-methyl groups. The main effect of introducing a carbonyl group at C-7 appears to be to rotate Me-8 back slightly, allowing more room for the 11-acetate. If ring c is a normal boat, then the predicted values for the coupling are in line with those found experimentally.

The di-deacetyl compound shows a 1H signal at δ 4.5 p.p.m. (J^* 4.5 c./sec.) and two superposed 1H resonances at δ 5.55 p.p.m. One of these is a singlet, representing 17-H, the other was not resolved at 60 Mc./sec. Oxidation gave a gum, which on treatment with acid gave an amorphous unsaturated ketone. This showed a pair of coupled doublets at δ 7.13, and 5.90 p.p.m. (J 10 c./sec.) which are characteristic of $\Delta^{1,2}$ -3-oxo-compounds. In the isomeric 1-oxo-compounds, the bands are typically near δ 6.3 and 5.7 p.p.m. This compound showed only one acetate group; the adjacent proton absorbed at δ 4.5 p.p.m. (J^* 4.5 c./sec.) and 15-H absorbed normally at δ 3.59 p.p.m. The compound is not, therefore, a 7-ketone, and it must be 11-ketogedunin (IV); the di-deacetyl compound is then the 1,7-diacetate (IIId). In the spectrum of 11-ketogedunin there is a sharp singlet at δ 3.66 p.p.m. which we consider represents 9-H; in

⁵ N. S. Ohochuku and D. A. H. Taylor, *J. Chem. Soc. (C)*, 1969, 864.

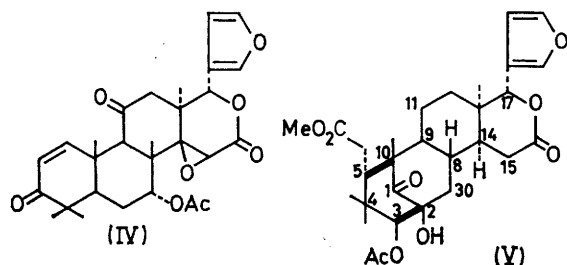
⁶ J. D. Connolly, R. McCrindle, K. H. Overton, and J. Feeney, *Tetrahedron Letters*, 1966, 22, 891.

⁷ D. A. H. Taylor, *Chem. Comm.*, 1967, 500.

⁸ D. A. H. Taylor, *Chem. Comm.*, 1968, 1172.

⁹ J. W. Powell, *J. Chem. Soc. (C)*, 1966, 1794.

11-ketocedrelone 9-H absorbs even further downfield at δ 4.05 p.p.m.¹⁰



The second new compound, m.p. 240°, had the molecular formula $C_{29}H_{38}O_9$. The u.v. spectrum showed only absorption attributable to a furan ring; the i.r.

hydroxy-group is at a bridgehead.¹¹ The suggested structure is supported by the results of double-resonance experiments at 100 Mc./sec. These show a triplet at δ 3.18 p.p.m. (J 6 c./sec.), coupled to a 2H doublet at δ 2.35 (J 6 c./sec.), which we assign to 5-H and 6-H. There was also a pair of doublets centred at δ 2.90 p.p.m. (J 6, 18 c./sec.). We consider the major coupling is to a pair of doublets, only one of which was visible at δ 2.73 p.p.m. (J 2 c./sec.), the other being concealed under other bands. Decoupling of the major coupling was not possible, as the bands were too close, but the minor coupling of both collapsed on irradiation at δ 1.37 p.p.m. We consider that these two pairs of doublets represent the two protons at C-15; hence the proton at δ 1.37 p.p.m. is 14-H. Irradiation at δ 1.37

N.m.r. spectra, in p.p.m. from Me_4Si (Spectra marked * are recorded at 100 Mc./sec., others at 60 Mc/s.)

	1-H	3-H	2-H	7-H	9-H	11-H	12-H	15-H	17-H	4 α -Me	4 β -Me	8-Me	10-Me	13-Me
11 β -Acetoxy-khivorin (IIb)	4.74 $J^ 5$	4.56 $J^* 5$		4.65 $J^* 6$	2.94 $J^* 4$	5.19 $w_1 18$	1.45 $J 15$	3.57	5.60	0.78	0.91	1.29	1.27	1.24
Deoxy-11 β -acetoxy-khivorin (III)	4.71 $J^* 6$	4.53 $J^* 6$		5.1	2.73 $J 5.5$	ca. 5		6.8	4.91	0.80	0.93	1.55	1.30	1.19
* 7-Ketone (IIc)	4.9 $J^* 6$	4.8 $J^* 6$	2.22		2.87	5.24 $J 6$ $w_1 3$	1.64	3.77	5.40	6.88	1.0	1.30	1.40	1.13
11-Ketogedunin (II)	7.13 $J 10$	5.90 $J 10$		4.50 $J^* 4.5$	3.66			3.58	5.53	1.05	1.05	1.35	1.45	1.25
* Fissinolide (I)	3.10 dd $J 10, 6$	4.96 d $J 10$	3.18 t $J 6.5$	2.38 d $J 6.5$	α 2.82 dd $J 15, 2.5$ β 1.96m	8-H	9-H 1.78 $J_{9-15}=3$	14-H	15-H α 3.76 dm $J 21$ β 3.42 d of t $J 21, 2, 3$	17-H 5.75	4 α -Me 0.81	4 β -Me 0.73	10-Me 1.16	13-Me 1.10
* Methyl 3 β -acetoxy-2-hydroxy-1-oxomeliacate (V)		5.05	3.18 t $J 6$	2.35 d $J 6$	A 2.51 d $J 10$ $w_1 3$ B 1.40m	1.75?		1.37 m $J 6, 2$	α 2.90 $J 18, 6$ β 2.73 $J 18, 2$	5.9	0.83	0.76	1.19	1.03
* Alcohol (VIIIa)				2.38 s $J 7$	A 2.82d $J 7$ B 1.59m				α 3.15 $J 17, 7$ β 2.25 $J 17, 8$	5.75	0.95	0.95	1.05	1.05
Acetate (VIIIb)		4.66		2.38 s						5.74	0.95	0.99	1.11	1.01

spectrum showed a hydroxy-group, a furan ring, and carbonyl groups similar to those in the spectrum of fissinolide. The n.m.r. spectrum showed the presence of a β -substituted furan, a methoxycarbonyl group, an acetate, and four tertiary methyl groups. There were also three downfield singlets at δ 5.9, 5.05, and 4.09 p.p.m. (OH, disappears with D_2O). The formula is that of a hydrate of fissinolide (I); if the downfield singlets represent 17-H and the proton adjacent to the acetate, it follows that if the acetate is at C-3, C-2 must be substituted, for which only the tertiary hydroxy-group is available. The compound might then be methyl 3 β -acetoxy-2-hydroxy-1-oxomeliacate (V). In agreement with this the compound is stable to concentrated hydrochloric acid, which suggests that the tertiary

p.p.m. also collapses the major coupling of a pair of broad singlets at δ 2.51 p.p.m. (J 10 c./sec., $w_1 = 3$ c./sec.). The region near δ 1.37 p.p.m. is complex, but irradiation at δ 2.9 p.p.m. (15a-H) reveals 14-H as a broad band at δ 1.36 p.p.m., while irradiation at δ 2.51 p.p.m. reveals a broad singlet at δ 1.40 p.p.m. We consider the doublets (J 10 c./sec.) at δ 2.51 and 1.40 p.p.m. represent the two protons at C-30, which are also coupled to 8-H. This last has not been certainly located, but is probably at δ 1.75 p.p.m.

The presence of two A/B pairs, each coupled to one further proton, is explained by the structure we suggest, but would, otherwise, appear difficult to explain.

Recently, we have assigned the tertiary methyl groups in fissinolide (I);⁵ we find that the 4 α -, 4 β -, 10-, and

¹⁰ C. W. L. Bevan, A. H. Rees, and D. A. H. Taylor, *J. Chem. Soc.*, 1963, 983.

¹¹ P. D. Bartlett and L. H. Knox, *J. Amer. Chem. Soc.*, 1939, **61**, 3184.

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13-methyl groups resonate at 49, 44, 70, and 66 c./sec. respectively, from tetramethylsilane at 60 Mc./sec. In the present compound, the methyl groups are little shifted and resonate at 50, 46, 71, and 72 c./sec.; we assign them correspondingly.

Alkaline hydrolysis gave a product m.p. 225°, oxidised to gummy mixtures by chromic acid in acetone, and by periodic acid. Reacetylation gave an acetate, m.p. 234–236°, isomeric with the natural product. The n.m.r. spectra of the two acetates differed only in detail. In the second isomer the proton adjacent to the acetate had moved slightly upfield to δ 4.66 p.p.m., and the methyl groups were at 57, 59, 67, and 61 c./sec. from tetramethylsilane at 60 Mc./sec. The 5-H triplet had disappeared, and the doublet representing the two protons at C-6 was reduced to a singlet. Decoupling of the 100 Mc./sec. spectrum of the alcohol showed the protons we ascribe to 15-H and 30-H in similar places to those in the original compound.

There are several possibilities for isomerisation. Epimerisation at C-3, as occurs on hydrolysis of swietenine,¹² has very little effect on the positions of the methyl group resonances,⁵ whereas in our second acetate 4 α - and 4 β -Me are shifted downfield by 7 and 15 or 10 and 13 c./sec., depending upon how they are assigned. Isomerisation of the type observed in cortisone¹³ could give (VI), (VII), or the 2-hydroxy-isomer of (VII). In (VI) we would expect the C-30 protons to be shifted downfield by the adjacent carbonyl; in (VII) the proton adjacent to

upfield; in the only known case [the compound (IX)¹²], the increment is 4 c./sec. (recalculated at 60 Mc./sec.), in the present case it is also 4 c./sec. Introduction of the 3-oxo-group in place of the 3 β -acetate is expected to move the 4 α - and 4 β -Me signals downfield; the average increments we find are 3 and 16 c./sec.,⁵ in reasonable agreement with the values of 7 and 15 c./sec. in the present case.

We consider therefore, that the hydrolysis product is (VIIIa). The postulated hydride shift can occur only in a 3 β -hydroxy-compound. Possibly the ready epimerisation of swietenine derivatives at C-3 has prevented this reaction being observed before, while in the present case the 2-hydroxy-group maintains the 3 β -configuration by preventing the de-aldolisation necessary for epimerisation.

EXPERIMENTAL

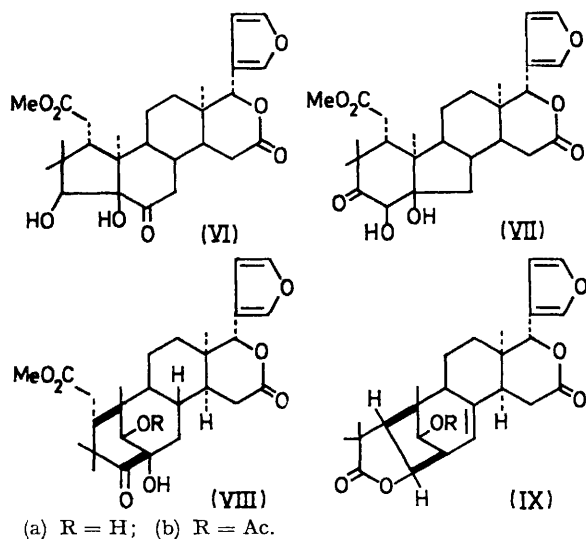
Optical rotations are for solutions in chloroform, determined at 20° with a Perkin-Elmer 141 polarimeter; mass spectra are recorded on a Perkin-Elmer-Hitachi RMU 6E instrument. N.m.r. spectra are in deuteriochloroform, 60 Mc./sec. spectra are recorded on a Varian A 56/60; 100 Mc./sec. spectra are recorded by Mr. P. Haylett of the University of East Anglia on a Varian HA 100.

Extraction of Seed.—*Khaya madagascariensis* seed (70 g.) collected from a tree near Majunga* was minced and extracted with light petroleum (b.p. 60–80°). The extract was chromatographed over neutral alumina. Elution with pentane–methylene chloride gave (i) fatty oil, (ii) fissionolide (35 mg.), m.p. 168° (n.m.r. and mass spectra identical with an authentic sample), and (iii) 3-deacetylkhivarin (375 mg.), m.p. 255° (n.m.r. and mass spectra identical with an authentic sample).

Extraction of Bark.—The bark of the above tree (2.5 kg.) was ground and extracted with light petroleum (b.p. 60–80°). The extract gave a solid precipitate (6.45 g.) but no crystalline material was obtained from this after chromatography. The root bark gave similar results.

Extraction of the Root.—Root wood from the above tree (4 kg.) was ground and extracted with light petroleum. The solid portion of the extract (14.8 g.) was chromatographed over neutral alumina. Pentane–methylene chloride mixtures eluted 11 β -acetoxylkhivarin (3.80 g.), m.p. 165°/218°/268° (from methylene chloride–methanol) (Found: C, 60.45; H, 7.1; 7.0%; M^+ , 644. $C_{34}H_{44}O_{12}$ requires C, 63.3; H, 6.9%); the n.m.r. spectrum showed the presence of methylene chloride; $[\alpha]_D^{20}$ –20°. Methylene chloride–methanol eluted methyl 3 β -acetoxyl-2-hydroxy-1-oxomeliacate (V) (2.63 g.), m.p. 238–240° (from methylene chloride–methanol) (Found: C, 65.3; H, 7.35%; M^+ , 530. $C_{29}H_{38}O_9$ requires C, 65.6; H, 7.2%); $[\alpha]_D^{20}$ –64°. Extraction of the timber gave similar results, but a rather lower yield.

Hydrolysis of 11 β -Acetoxylkhivarin.—The acetate (IIb) (5.0 g.) in boiling methanol (800 ml.) was treated with refluxing aqueous potassium hydroxide (125 ml.; 2N) for 10 min. The solution was acidified with dilute sulphuric acid (125 ml.; 2N) and the methanol was evaporated off; the residue was diluted with methylene chloride and water, and the methylene chloride extract was evaporated to dryness. The residue was chromatographed on neutral



the acetate would be similarly shifted downfield. Neither of these effects is observed. We consider the observations are best explained by supposing a hydride transfer from C-3 to C-1, so that the second acetate is (VIIIb). The introduction of a 1-acetoxy-group in place of the 1-carbonyl would be expected to shift the 10-Me signal

* Herbarium specimens are preserved at Kew as DAHT 251.

¹² J. D. Connolly, R. Henderson, R. McCrindle, K. H. Overton, and N. S. Bhacca, *J. Chem. Soc.*, 1965, 6935.

¹³ L. F. and M. Fieser, 'Steroids,' Reinhold, New York, 1959, p. 582.

alumina (Grade V) with methylene chloride-pentane as eluant. The first fractions gave 11 β -acetoxykhivirin (540 mg.); subsequent fractions (300 mg.) did not crystallise, but appeared to be pure (n.m.r.). Methylene chloride eluted 1 α ,7 α -diacetoxy-3 α ,11 β -dihydroxy-14 β ,15 β -epoxymeliacolid (IIId) (1.18 g.), which crystallised from methanol as needles m.p. 275–280° (Found: C, 64.1; H, 7.4. C₃₀H₄₀O₁₀ requires C, 64.3; H, 7.2%). Methylene chloride-methanol eluted noncrystalline material which was not investigated.

14 β ,15 β -Epoxy-7-oxo-1 α ,3 α ,11 β -triacetoxymeliacolid (IIc).—The above amorphous fraction (300 mg.) was dissolved in acetone and oxidised with chromic acid (8N; 0.15 ml.). The solution was diluted with methylene chloride and water and the organic layer was evaporated to dryness; the residue crystallised from methanol to give the ketone (IIc) (190 mg.) as plates, m.p. 268–270° (Found: C, 63.8; H, 6.9%; M⁺ 600. C₃₂H₄₀O₁₁ requires C, 64.0; H, 6.7%); $[\alpha]_D^{20}$ –46°.

11-Ketogedunin.—The dideacetyl compound (IIId) (860 mg.), dissolved in acetone, was oxidised with chromic acid (0.325 ml.; 8N) and the product, dissolved in methanolic sulphuric acid (60 ml.; N), was heated under reflux for $\frac{1}{2}$ hr. The product (IV), isolated with methylene chloride, remained amorphous, but appeared to be pure (t.l.c., n.m.r.). Hydro-

genation over platinum oxide in methanol gave the dihydro-derivative, which also failed to crystallise.

11 β -Acetoxydeoxykhivirin.—This was prepared by chromous chloride reduction.¹⁴ The product had m.p. 292–294° (insoluble in methanol) (Found: C, 64.6; H, 7.3. C₃₄H₄₄O₁₁ requires C, 64.95; H, 7.05%); $[\alpha]_D^{20}$ +47.5°.

Alkaline Hydrolysis of the Acetate (V).—The acetate (200 mg.) and potassium hydroxide (200 mg.) in methanol (20 ml.) were heated under reflux for $\frac{1}{2}$ hr. The solution was acidified with dilute hydrochloric acid and the product, extracted with methylene chloride and crystallised from methanol, had m.p. 225° (Found: C, 66.1; H, 7.5%; M⁺ 488. C₂₇H₃₆O₈ requires C, 66.4; H, 7.4%); $[\alpha]_D^{20}$ –100°. Acetylation gave the isomeric acetate (VIII), m.p. 234–236° (Found: C, 65.7; H, 7.6%; M⁺ 530. C₂₈H₃₈O₉ requires C, 65.6; H, 7.2%); $[\alpha]_D^{20}$ –76°.

The author is grateful to H. M. Consul in Tananarive and to the Chef de Service Provincial des Eaux et des Forêts, Majunga, for assistance in collecting material.

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¹⁴ C. W. L. Bevan, T. G. Halsall, M. N. Nwaji, and D. A. H. Taylor, *J. Chem. Soc.*, 1962, 768.