389. *The Isolation and Structure of Gafrinin, a Sesquiterpenoid Lactone from* Geigeria africana.

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A new sesquiterpenoid lactone, gafrinin, $C_{17}H_{24}O_5$, has been isolated from *Geigeria africana* Gries. It contains an acetoxy-group, a hydroxy-group, and two double bonds. It is monocyclic and structure (I) is proposed.

THREE crystalline compounds, vermeerin, geigerin, and geigerinin, have been isolated from *Geigeria aspera* Harv.[1,2] and the structures of geigerin [3] and geigerinin [2] have been determined.

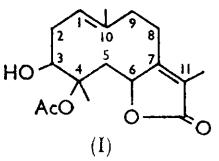
A new, slightly bitter, sesquiterpenoid acetoxy-lactone, gafrinin, $C_{17}H_{24}O_5$, has been isolated from *G. africana* Gries., the species of " vermeerbos " responsible for " vermeersiekte " (vomiting disease) among sheep in the N.W. Cape Province. Structure (I) is proposed for gafrinin for the reasons advanced below.

Gafrinin contains a readily reducible trisubstituted double bond (infrared maximum at 813 cm.$^{-1}$). Dihydrogafrinin still shows the ultraviolet absorption of an $\alpha\beta$-unsaturated γ-lactone and must contain a double bond. Gafrinin thus has two double bonds and must be monocyclic, belonging to the group of ten-membered-ring sesquiterpenoid lactones occurring in the Compositæ.[4,5] In common with the other members of this group, cited by Herout, Soucek, and Šorm,[5] gafrinin also gives chamazulene on dehydrogenation.

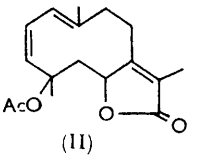
[1] Rimington and Roets, *Onderstepoort J. Vet. Sci.*, 1936, **7**, 485.
[2] De Villiers, *J.*, 1959, 2412.
[3] Barton and Levisalles, *J.*, 1958, 4518.
[4] Barton and de Mayo, *J.*, 1957, 150.
[5] Herout, Soucek, and Šorm, *Chem. and Ind.*, 1959, 1069.

Structures based on ring systems containing either a six-membered ring or the seven-membered ring of xanthinin [6] are excluded by the impossibility of accommodating the various substituents on such ring systems. The structure of xanthinin type is also eliminated as xanthinin does not give chamazulene on dehydrogenation.

C-Methyl determinations indicate four *C*-methyl groups; therefore, in addition to the acetyl group, there are the usual three *C*-methyl groups in the molecule, and primary oxygen functions and vinylidene groups are excluded. The double bond conjugated to the



(I)



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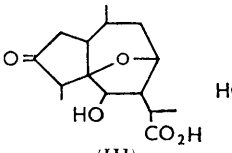
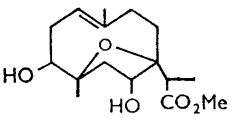


(III)



(IV)

lactone must be in the 7,11-position, as in (I). The ultraviolet absorption of dihydrogafrinin ($\lambda_{max.}$ 219 mμ, log ε 3·84) is in agreement with this formulation,[7] as is the resistance of this double bond to hydrogenation. The high absorption of gafrinin at lower wavelength ($\lambda_{max.}$ 205 mμ, log ε 4·05) is due to the additional contribution of the trisubstituted double bond.

Oxidation of dihydrogafrinin with chromium trioxide gave deacetyldehydrodihydrogafrinin, containing a ketone group and a hydroxyl group stable to oxidation. As the hydroxyl group in gafrinin is readily acetylated, it is unlikely to be tertiary, and must thus be the precursor of the ketone group in deacetyldehydrodihydrogafrinin; while the hydroxyl group resistant to oxidation must be formed by the hydrolysis of the acetoxy-group present in gafrinin. Preparation of the 2,4-dinitrophenylhydrazone of this ketone caused elimination of the hydroxyl group; the ultraviolet spectrum of this derivative was in agreement with that of a substituted αβ-unsaturated phenylhydrazone.[8] Deacetyldehydrodihydrogafrinin reduced one mol. of periodate, indicating that the ketone and the hydroxyl groups are vicinal.

On sublimation from alumina in a high vacuum, gafrinin lost one mol. of water, to give anhydrogafrinin (II). The ultraviolet spectrum of this compound ($\lambda_{max.}$ 226 mμ, log ε 4·3) is the sum of the absorptions due to the αβ-unsaturated lactone chromophore ($\lambda_{max.}$ 219 mμ, log ε 3·8) and that of a conjugated diene system ($\lambda_{max.}$ 230 mμ, log ε 4·1). The formation of this diene by dehydration of the hydroxyl group requires the hydroxyl group to be on $C_{(3)}$ and the trisubstituted double bond to be in the 1,10-position.

Treatment of gafrinin with sodium methoxide in dry methanol yielded methyl deacetylgafrinate, which did not show the ultraviolet absorption due to the αβ-unsaturated ester. This was first considered to be due to the formation of a ketone grouping on the site of the hydroxyl group that had been lactonized, but no absorption of such a carbonyl group could be detected in either the ultraviolet or the infrared spectrum. Methyl deacetylgafrinate gave a diacetate which contains no further hydroxyl group. The tertiary hydroxyl group, on the site of the original acetate group in gafrinin, had thus also been lost in the formation of methyl deacetylgafrinate. If the formation of a five-membered-ring ether, similar to that advanced by Barton and Levisalles [3] for allogeigeric acid (III), is postulated, it explains both the disappearance of one hydroxyl group and the change in the ultraviolet spectrum. Methyl deacetylgafrinate thus has structure (IV). It reduced two mol. of periodate, which is in agreement with the behaviour of allogeigeric acid which reduced one mol.[9]

The position of the lactonized hydroxyl group in gafrinin remained to be determined.

[6] Dolejs, Herout, and Šorm, *Coll. Czech. Chem. Comm.*, 1958, **23**, 504.
[7] Büchi and Rosenthal, *J. Amer. Chem. Soc.*, 1956, **78**, 3860.
[8] Braude and Jones, *J.*, 1945, 498.
[9] Perold, *J.*, 1957, 47.

Methyl deacetylgafrinate has two hydroxyl groups, one at position 3, the other at position 6 or 8. Attempted dehydration of methyl deacetylgafrinate led to the loss of only the 3-hydroxyl group and, although this result confirmed the formation of the diene system postulated for anhydrogafrinin, the failure of methyl deacetylgafrinate to yield, by further dehydration, either a diene and an isolated double bond or a triene did not enable the position of the other hydroxyl group to be established.

Frommodels it was found that methyl deacetylgafrinate would give an isopropylidene derivative only if the remaining hydroxyl group was at position 6. Under the usual conditions methyl deacetylgafrinate readily gave such an isopropylidene derivative, establishing the position of this hydroxyl group as 6. Methyl deacetylgafrinate thus has structure (IV), and gafrinin has structure (I).

Experimental

Ultraviolet spectra were measured for 96% EtOH solutions in a Unicam S.P. 500 spectrophotometer. The infrared spectra were measured in an Infracord spectrophotometer for potassium bromide discs. $[\alpha]_D$ were determined for EtOH solutions. M. p.s are corrected.

Geigeria africana was obtained from the Agricultural Research Station, Koopmansfontein, through the courtesy of the Officer-in-Charge, Mr. J. P. Ebersöhn.

Gafrinin.—Ground, air-dried *G. africana* (17 kg.) was extracted at room temperature, first with hexane, and then twice with 96% ethanol. The ethanol extracts were combined and their volume was reduced to 8 l. An equal volume of water was added, followed by saturated aqueous lead acetate solution (3 l.). After filtration, the excess of lead was removed with potassium dihydrogen phosphate. The clear solution was extracted repeatedly with hexane until no more colour was removed. The alcohol solution was then boiled with charcoal and filtered. The yellow-brown filtrate was diluted with water and extracted with chloroform until colourless. The chloroform extracts were combined and washed with water, and the solvent was removed, giving a viscous brown tar (200 g., 1·2%).

This tar (40 g.) was chromatographed on a column of cellulose powder (2 kg.) impregnated with formamide,[10] with 1 : 1 hexane–benzene as eluant. The chromatogram was controlled by spotting the fractions on paper, impregnating the paper with formamide and developing the spots in hexane–benzene. Fractions, giving a brown spot, R_F 0·19, on being heated at 100° for 3 min. after spraying with the Carr–Price reagent, were combined. After removal of the eluting solvents, the residue was dissolved in chloroform and washed with water, and the chloroform removed by distillation. The residue crystallized from acetone–ether, and recrystallization from the same solvents gave *gafrinin* (2·3 g. from 40 g. of crude extract, 6%) as fine white needles, m. p. 110°, $[\alpha]_D^{28}$ −16·1° (*c* 5·2), $\lambda_{max.}$ 205 mμ (log ε 4·05), $\nu_{max.}$ 3509 (OH), 1751 (αβ-unsaturated γ-lactone), 1701 and 1250 (OAc), and 813 cm.$^{-1}$ (trisubstituted C=C) (Found: C, 66·3; H, 7·9; *C*-Me, 20·1. $C_{17}H_{24}O_5$ requires C, 66·2; H, 7·9; 4*C*-Me, 19·4%).

Gafrinin gave a yellow colour with tetranitromethane, and, on hydrogenation over palladium–calcium carbonate in methanol, absorbed 1·0 mol. of hydrogen, to give *dihydrogafrinin* as an oil, n_D^{20} 1·4981, $[\alpha]_D^{18}$ −10·8° (*c* 4), $\lambda_{max.}$ 219 mμ (log ε 3·84) (Found: C, 65·5; H, 8·8. $C_{17}H_{26}O_5$ requires C, 65·8; H, 8·4%).

With acetic anhydride–perchloric acid, gafrinin gave a yellow oil which was purified by chromatography on acid-washed alumina in ether, followed by distillation, to give *gafrinin acetate*, b. p. 230°/0·5 mm., n_D^{20} 1·5022, $[\alpha]_D^{18}$ +26·5° (*c* 4·4) (Found: C, 65·4; H, 7·5. $C_{19}H_{26}O_6$ requires C, 65·1; H, 7·4%).

Dehydrogenation of Gafrinin.—Dihydrogafrinin (2·5 g.) was dissolved in aqueous potassium hydroxide, and the solution was filtered, then acidified and extracted with chloroform. The solvent was removed by distillation and 30% palladized charcoal (0·75 g.) was added to the residue. After 1 hour's heating at 330° in nitrogen the mixture was extracted with hexane and the heating repeated. The hexane extracts were combined and chromatographed repeatedly on alumina with hexane as eluant, to give an intensely blue oil (35·5 mg.). With 1,3,5-trinitrobenzene, black needles (12 mg.) were obtained, which, on recrystallization from ethanol, had m. p. 132—133° alone or mixed with the chamazulene adduct.

[10] Enslin, Rehm, and Rivett, *J. Sci. Food Agric.*, 1957, **8**, 673.

Deacetyldehydrodihydrogafrinin.—Dihydrogafrinin (1·2 g.) in acetone was oxidized by the gradual addition of 8N-chromium trioxide in dilute sulphuric acid [11] at room temperature. After dilution with water, the mixture was extracted with ether, and the ether extracts were combined and dried (Na_2SO_4). The residue obtained on removal of the solvent was chromatographed on acid-washed alumina (15 g.) in ether. The first 25 ml. eluted contained most of the material (0·9 g.), which was distilled to give *deacetyldehydrodihydrogafrinin*, b. p. 210°/1 mm., n_D^{20} 1·5009, $[\alpha]_D^{18}$ −14·3° (*c* 4), $\lambda_{max.}$ 219 (log ε 3·8) and 290 mμ (log ε 2·7), $\nu_{max.}$ 3390 (OH), 1748 (γ-lactone) and 1724 cm.$^{-1}$ (medium-ring ketone) (Found: C, 67·5; H, 8·1. $C_{15}H_{22}O_4$ requires C, 67·6; H, 8·3%). It reacted with 2,4-dinitrophenylhydrazine with simultaneous dehydration of the tertiary hydroxyl group, to give *anhydrodeacetyldehydrodihydrogafrinin 2,4-dinitrophenylhydrazone* as red crystals, m. p. 229—230°, $\lambda_{max.}$ (in $CHCl_3$) 255, 305, and 393 mμ (log ε 4·18, 4·17, and 4·52 respectively) (Found: C, 58·3; H, 5·4; N, 12·6. $C_{21}H_{24}N_4O_6$ requires C, 58·9; H, 5·7; N, 13·1%).

Methyl Deacetylgafrinate.—Gafrinin (0·5 g.) was dissolved in dry methanol (50 ml.) containing sodium methoxide (0·2 g.). After 3 days at 4°, the solution was neutralized with solid carbon dioxide and most of the methanol removed under a vacuum. The residue was diluted with water and extracted with chloroform. The chloroform extracts were dried, the solvent was removed, and the residue recrystallized from acetone–ether, to give *methyl deacetylgafrinate* as white crystals (0·35 g.), m. p. 94—95°, $\lambda_{max.}$ 205 mμ (log ε 3·84), $[\alpha]_D^{18}$ −40° (*c* 1) (Found: C, 64·5; H, 8·6. $C_{16}H_{26}O_5$ requires C, 64·4; H, 8·7%).

With acetic anhydride–pyridine, methyl deacetylgafrinin yielded a product which on chromatography on acid-washed alumina in ether gave a clear oil, n_D^{20} 1·4856, which slowly crystallized to give *methyl diacetyldeacetylgafrinate*, m. p. 49·5—50·5°, $[\alpha]_D^{20}$ −62·5° (*c* 3·2) $\lambda_{max.}$ 205 mμ (log ε 3·9) (Found: C, 62·5; H, 8·0. $C_{20}H_{30}O_7$ requires C, 62·8; H, 7·9%).

When shaken with dry acetone and anhydrous copper sulphate for 4 days at 20°, methyl deacetylgafrinin gave the *isopropylidene derivative* as an oil, n_D^{20} 1·4927, $[\alpha]_D^{20}$ −23·2° (*c* 2·8), $\lambda_{max.}$ 203 mμ (log ε 3·7) (Found: C, 67·4; H, 9·1. $C_{19}H_{30}O_5$ requires C, 67·4; H, 8·9%).

Anhydrogafrinin.—Gafrinin (100 mg.), mixed with alumina (200 mg.), was heated at 180°/0·2 mm. During 2 hr. a sublimate (34 mg.) was collected, chiefly as a viscous oil. Crystallization from methanol gave *anhydrogafrinin*, m. p. 55—56°, $\lambda_{max.}$ 226 mμ (log ε 4·3) (Found: C, 70·3; H, 7·7. $C_{17}H_{22}O_4$ requires C, 70·5; H, 7·6%).

Similar treatment of methyl deacetylgafrinin gave *methyl anhydrodeacetylgafrinate* as an oil, n_D^{20} 1·5023, $[\alpha]_D^{20}$ −5·2° (*c* 3·1), $\lambda_{max.}$ 231 mμ (log ε 4·0) (Found: C, 68·6; H, 8·3. $C_{16}H_{24}O_4$ requires C, 68·5; H, 8·6%).

Periodate Oxidations.—Gafrinin, deacetyldehydrodihydrogafrinin, and methyl deacetylgafrinate (50 mg.) were severally dissolved in methanol (1 ml.), 0·2M sodium periodate (2 ml.) was added to each, and the solutions were made up to 5 ml. with water. After 24 hr. the periodate consumed was determined in the usual way. Gafrinin consumed no periodate, deacetyldehydrodihydrogafrinin consumed 1·11 mol., and methyl deacetylgafrinate consumed 2·06 mol.

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[11] Bowers, Halsall, Jones, and Lemin, *J.*, 1953, 2548.