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GRIESENIN AND DIHYDROGRIESENIN, TWO NEW SESQUITERPENOID LACTONES FROM *GEIGERIA AFRICANA* GRIES—I

STRUCTURES

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Abstract—Griesenin and dihydrogriesenin, two new sesquiterpenoid lactones have been isolated from *Geigeria africana* Gries. Structures I and II, respectively, are proposed for these lactones based on chemical and spectroscopic evidence.

SEVERAL crystalline sesquiterpenoid lactones have been isolated from *Geigeria aspera* Harv. and the related species, *Geigeria africana* Gries and their structures determined. They are geigerin,[1,2] geigerinin,[3] vermeerin,[1,4] and the acetoxy lactone, gafrinin.[5-7] In South Africa the Geigeria species are commonly known as "vermeerbos" and are responsible for "vermeersiekte" (vomiting disease) among sheep.

Griesenin, I, $C_{15}H_{16}O_4$, m.p. 196–197·5°, $[\alpha]_D$ +284° (*c*, 0·61 in EtOH), exhibited IR (1770 and 1663 cm^{-1}) and UV spectra (λ_{max} 208 mμ, ε 15,250) characteristic for an α,β'-unsaturated γ-lactone of the type found frequently in other sesquiterpenoid lactones[3,4,6] from Geigeria species. This is supported by the NMR spectrum of griesenin which shows two vinyl resonances at τ = 3·74 (doublet, *J* = 2·0 Hz) and τ = 4·31 (doublet, *J* = 1·6 Hz) usually observed for an exocyclic methylene group in conjugation with a γ-lactonic carbonyl.[6-8] These resonances exhibit allylic couplings to the proton on C-7[7,8] while the coupling between the two methylene protons is not resolved.

Ozonolysis of griesenin produced formaldehyde and treatment of griesenin with diazomethane gave a crystalline pyrazoline.[9] Griesenin reacted with sodium methoxide in dry methanol to give the Michael adduct III in which methanol had added over the exocyclic methylene group as described by Herz *et al.*[10] The doublets of the exocyclic methylene protons had disappeared in the NMR spectrum and new resonances for the O-methyl (singlet, τ = 6·64) and O-methylene groups (AB-part of an ABX-system centred around τ = 6·35) were observed.

The multiplicity of the NMR signals of the angular protons H-7 and H-8 showed their proximity to the methylene groups, and thereby established partial structure A for griesenin.

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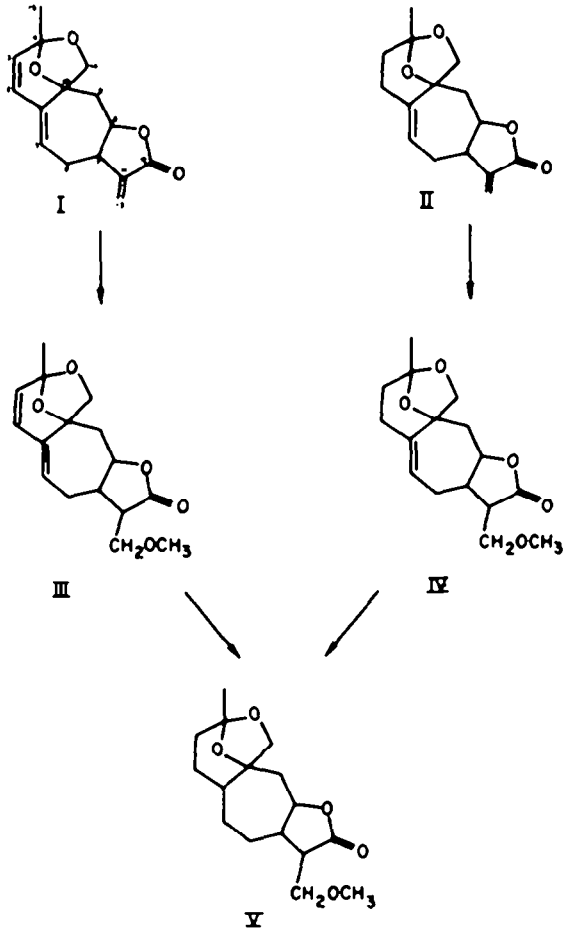
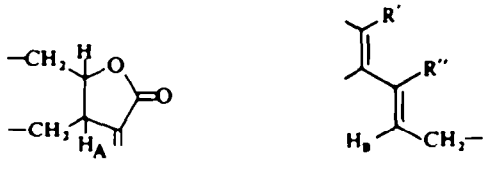
SCHEME 1



The remaining two oxygen functions of griesenin were not present as CO groups since the IR spectrum exhibited no other CO absorption than that of the unsaturated γ-lactone while the UV showed no absorption between 270 and 330 mμ. Griesenin did not react with 2,4-dinitrophenylhydrazine. The IR spectrum also gave no indication of OH absorption and griesenin could not be acetylated under various conditions. The NMR spectrum of griesenin in $CDCl_3$ also gave no indication of an OH



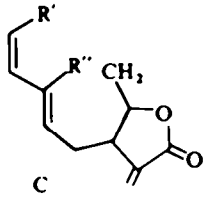
proton which could be exchanged by shaking with D_2O. A lactone titration indicated the presence of only one saponifiable group and thereby eliminated the presence of a second γ-lactonic function. A peroxide function was also absent since no iodine was detected (starch paper) when griesenin was treated with potassium iodide in acid medium. The two remaining oxygen functions were shown to be present in a ketal system of the type depicted in I (*vide infra*).

The IR spectrum (in KBr) of griesenin exhibited bands at 1648, 1608 and 970 cm^{-1} characteristic of a conjugated diene system.[11] The band at 970 cm^{-1} (C—H out-of-plane bending) furthermore suggested that the diene was *trans*. This was supported by the UV spectrum of griesenin which showed a strong absorption maximum at 237 mμ (ε 17,900).

The NMR spectrum of griesenin shows five protons in the olefinic region, two of which belong to the exocyclic methylene group. There are consequently three protons on the *trans*-diene grouping. Two of them form an AB-system (τ = 4·00 and 4·17) with a typical *cis*-coupling[12] (J = 9·4 Hz). The remaining olefinic proton (H-5) is a quartet at τ = 4·35 with couplings of 3·3 and 9·1 Hz to a neighbouring methylene group (C-6). There is only a small long-range coupling between H-5 and one of the olefinic protons (H-2) on the diene system (J = 0·7 0·8 Hz).

These results proved the presence of a *trans*-diene with one of the double bonds trisubstituted and the other disubstituted as shown in the partial structure B.

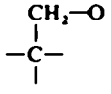
Detailed NMR analyses (see following paper) established that the vinyl proton H_B on the trisubstituted double bond in structure B and the angular proton H_A in structure A are flanked by the same methylene group. This information now leads to a combination of fragments A and B to give partial structure C for griesenin.



C

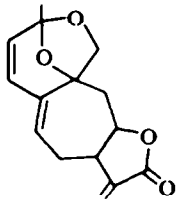
The Me region of the NMR spectrum of griesenin exhibits only one unsplit 3-proton signal at τ = 8·44. The low τ-value and the lack of fine structure suggest that the Me is attached to a quarternary C atom bearing at least one O atom as expressed in fragment D. The NMR spectrum furthermore shows an AB-quartet at τ = 6·12 and 6·23 which is assigned to a CH_2O-grouping. The methylene group must be attached to a quarternary C atom (fragment E) as the AB-pattern shows no further splitting.

D

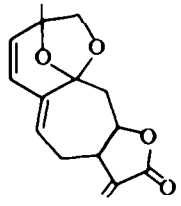


E

To derive the structure of griesenin from the fragments C, D and E we will have to take all known facts into consideration. It has been shown by elimination that two of the O atoms are present as ether links. From the sum formula, functional groups and number of double bonds follows therefore that griesenin must have a monocarbocyclic structure. It can be concluded from the NMR splitting pattern that (i) R' in partial structure C must be a quarternary C atom and (ii) the CH_2-group in C must also be attached to a fully substituted carbon. These quarternary C atoms have to be identical to those in fragments D and E (sum formula). There are various ways of linking these fragments. Those having fragment E in an epoxide or methylene dioxy arrangement are ruled out by the chemical shift of the methylene protons[13] and by the magnitude of the geminal coupling constant.[12,14] There emerge finally two possible structures F and G.



F



G

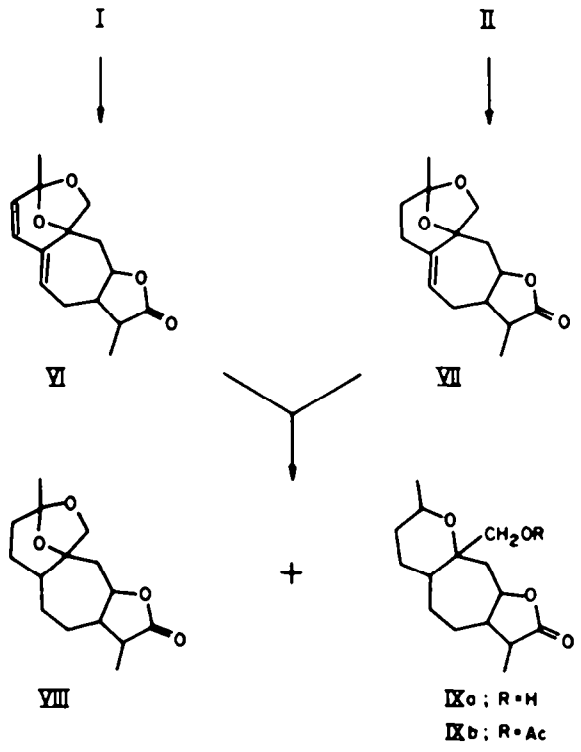
Reductive cleavage of one of the ether links provided proof that griesenin has structure F.

Various procedures[15-18] for hydrolysing the ketal to a ketone were applied unsuccessfully. However, reduction[19] of the exocyclic methylene group of griesenin I with $NaBH_4$ gave the reduction product VI which upon hydrogenation in ethyl acetate over Pt catalyst at 60° and under 8 atm pressure, gave hexahydro-griesenin VIII as the main product (80% yield) and an alcohol IXa (ca. 10% yield). The latter was formed by reductive cleavage of one of the ether rings. The alcohol showed strong IR absorption at 3500 (OH) and 1760 cm^{-1} (saturated γ-lactone). A mass spectrum of the alcohol IXa showed no molecular ion peak but a strong M-31 peak due to the facile loss of the CH_2OH fragment. Acetylation of the alcohol under mild conditions gave the acetate IXb.

The alcohol IXa and its acetate IXb both showed two secondary Me groups in the NMR spectrum (IXa: τ_{13} = 8·88; d, J = 7·0, τ_{15} = 8·85; d, J = 6·0; IXb: τ_{13} = 8·89; d, J = 7·0; τ_{15} = 8·89; d, J = 5·6 Hz). There are furthermore four protons on C atoms bearing oxygen functions (C-4, C-8 and C-14). The two complex multiplets in the NMR spectrum of IXa at τ = 6·36 and 5·39 are assigned to the protons H-4 and H-8, respectively. The C-14 methylene protons form an AB-pattern with τ-values of 6·00 and 6·68. In the corresponding acetate (IXb) no significant changes of the first two proton signals are observed (τ_4 = 6·33; τ_8 = 5·40), while the C-14 protons shift downfields to τ = 5·47 and 5·99.

These data prove that a C-4 oxygen bond has been cleaved (two secondary Me's) and that a CH_2OH group has been formed (loss of CH_2OH in the MS, downfield shift of the CH_2 protons on acetylation). This excludes structure G conclusively.

SCHEME 2



Dihydrogriesenin, II, $C_{15}H_{18}O_4$, m.p. 139·5–140·5°, $[\alpha]_D$ +92° (*c*, 0·72 in EtOH) occurs together with griesenin in *Geigeria africana* Gries and can be separated from griesenin by chromatography on formamide impregnated cellulose. This compound only differs from griesenin in that it has, apart from the conjugated exocyclic double bond only one other trisubstituted double bond instead of the *trans*-diene system of griesenin. This fact was evident from the UV spectrum (λ_{max} 206 mμ, ε 17,900) and NMR spectrum which exhibited, apart from the methylene doublets, only one other vinylic proton signal at τ = 4·46. That compound II is in fact a dihydro derivative of griesenin was indicated as follows: Treatment of II with sodium methoxide gave the Michael adduct IV which, upon hydrogenation over Pd–$CaCO_3$ in EtOH, absorbed 1·01 mole of hydrogen to give the saturated compound V which was in all respects identical (m.p., mixed m.p., TLC and IR) with the corresponding tetrahydro derivative of griesenin obtained from griesenin by a similar route.

EXPERIMENTAL

M.ps are uncorrected. UV spectra and $[\alpha]_D$ refer to EtOH, IR spectra to $CHCl_3$ and NMR spectra to $CDCl_3$ solns, unless otherwise stated. IR spectra were recorded on Perkin Elmer models 21 and 237 spectrometers, UV spectra on a Unicam Model S.P. 800 spectrometer and NMR spectra on Varian A-60 and HA-100 spectrometers. Chemical shifts were measured on the τ-scale relative to TMS as internal standard (τ = 10·0); τ-values are estimated to be accurate to ±0·01 ppm, coupling constants to ±0·2 Hz. Mass spectra were recorded on a MS-9 spectrometer

TLC was carried out on silica gel plates using $CHCl_3$–MeOH (19:1) as solvent system. The spots were developed with the vanillin-phosphoric acid reagent or with 0·5% $KMnO_4$ in saturated copper acetate.

Geigeria africana was obtained from the Rietondale Experimental Farm, Pretoria, through the courtesy of Dr. T. Terblanche of Onderstepoort

Extraction and isolation of griesenin and dihydrogriesenin Ground, air-dried *G. africana* (34 kg) was extracted several times with hot, 96% EtOH The extract was concentrated to 12 l. and water (2·5 l.) added. Chlorophyll and fats were extracted with hexane and the aqueous residue then treated with a hot soln of basic lead acetate (1·5 kg in 2 l water) The lead acetate ppt was removed by centrifugation and the clear aqueous layers thoroughly extracted with $CHCl_3$. This solvent was removed *in vacuo* and the tarry residue (370 g) was taken up in formamide from which the less polar sesquiterpenoids were then extracted with benzene. Evaporation of the benzene *in vacuo* left a tarry residue (140 g) which was chromatographed in portions of 45 g on cellulose (1·5 kg) impregnated with a 40% soln of formamide in acetone The chromatograms were controlled by TLC of the individual fractions.

Hexane–benzene (9:1) eluted fractions which gave green spots (R_f 0·7) with the vanillin-phosphoric acid spray reagent on chromatoplates. These combined fractions were evaporated to dryness and the residue (2·33 g) crystallized several times from chloroform ether to give colourless crystals of *dihydrogriesenin* II (750 mg), m.p. 139·5 140·5°, $[\alpha]_D$ + 92° (*c* 0·72), λ_{max} 206 mμ (ε 17,900), ν_{max} 1760 (αβ'-unsat.-γ-lactone), 1665 (double bond), 1388, 1270, 950 and 864 cm^{-1} (Found: C, 68·8; H, 6·9; M (mass spect.) 262. $C_{15}H_{18}O_4$ requires: C, 68·7; H, 6·9%; M, 262).

Hexane–benzene (9:1) subsequently eluted fractions which gave characteristically pink spots (R_f 0·7) with the vanillin-phosphoric acid spray reagent on chromatoplates. These fractions were combined, evaporated down and the residue (3·56 g) crystallized from $CHCl_3$–ether to give *griesenin* as colourless needles (1·59 g), m.p. 196 197·5°, $[\alpha]_D$ + 284° (*c* 0·61), λ_{max} 208 mμ (ε 15,250) and 237 mμ (ε 27,900), ν_{max}^{KBr} 1770 (αβ'-unsat. γ-lactone), 1663, 1648 and 1608 (double bonds), 970, 866 and 845 cm^{-1}. (Found: C, 69·2; H, 6·1; M (mass spect.) 260. $C_{15}H_{16}O_4$ requires: C, 69·2, H, 6·2%; M, 260).

Pyrazoline derivative[9] *of griesenin.* An ethereal soln of diazomethane prepared from nitrosomethyl urea (350 mg) was added to a suspension of griesenin (100 mg) in abs ether (100 ml) and left in the cold for three days. The solvent was removed and the residue crystallized from acetone–ether to give colourless *crystals*, m.p. 135–137°, $[\alpha]_D$ + 376° (*c* 0·37) (Found: C, 63·3; H, 6·0; N, 9·7. $C_{16}H_{18}O_4N_2$ requires: C, 63·6, H, 6·0; N, 9·3%)

Ozonolysis of griesenin Griesenin (100 mg) in AcOH (25 ml) was treated with a stream of O_2 containing 2 mg of O_3/min for 90 min. The mixture was steam-distilled into aqueous dimedone. Upon concentration, a ppt (20 mg) was formed which had m.p. 191° alone or mixed with the formaldehyde derivative of dimedone.

Treatment of griesenin with sodium methoxide in methanol. A soln of Na (700 mg) in dry MeOH (25 ml) was added to a soln of griesenin (500 mg) in dry MeOH (25 ml) and left at 3° for 4 days The soln was acidified with HCl to pH_2 and then extracted with $CHCl_3$ (6 × 50 ml) The latter was washed with water, dried over Na_2SO_4 and evaporated to dryness. The residue was crystallized from acetone–ether to give fine needles (96 mg) of the *Michael adduct* III, m.p. 146–147·5°, $[\alpha]_D$ + 214 (*c* 0·43), λ_{max} 238 mμ (ε 15,900), ν_{max} 1775 (sat. γ-lactone), 1655 and 1615 (double bonds), 1390, 866 and 846 cm^{-1}. (Found: C, 65·6; H, 6·9, M (mass spect.) 292. $C_{16}H_{20}O_5$ requires: C, 65·7; H, 6·9%; M, 292).

Treatment of dihydrogriesenin with sodium methoxide in MeOH. Dihydrogriesenin (II, 500 mg) was treated with MeONa in dry MeOH as described above. The crude product was crystallized from ether–hexane to give needles of the *Michael adduct* IV, m.p. 92·5 93·5°, $[\alpha]_D$ + 52° (*c* 0·46), λ_{max} 211 mμ (ε 3500), ν_{max} 1774 (sat. γ-lactone), 1648 (trisubst. double bond), 1392, 956 and 866 cm^{-1}. (Found: C, 65·4; H, 7·4; M (mass spect.) 294. $C_{16}H_{22}O_5$ requires: C, 65·3, H, 7·5%; M, 294)

Hydrogenation of the Michael adduct III *of griesenin* The Michael adduct III (119 mg) absorbed 1·96 moles H_2 upon hydrogenation over 5% Pd·$CaCO_3$ (149 mg) in 96% EtOH The catalyst was filtered off, the EtOH removed *in vacuo* and the residue crystallized from acetone to give *needles*, V, m.p. 136 138°, $[\alpha]_D$ + 22° (*c* 0·49), ν_{max} 1778, 1393, 1042, 1030 and 987 cm^{-1} (Found: C, 65·1, H, 8·1. $C_{16}H_{24}O_5$ requires: C, 64·8; H, 8·2%)

Hydrogenation of the Michael adduct IV *of dihydrogriesenin* The Michael adduct IV (121 mg) absorbed 1·01 moles H_2 when hydrogenated as above. Crystallization of the product from acetone afforded *needles*, V, m.p. 137·5 139°, $[\alpha]_D$ + 19° (*c* 0·56), ν_{max} 1778, 1393, 1042, 1030 and 987 cm^{-1}. (Found: C, 65·0; H, 8·2. $C_{16}H_{24}O_5$ requires: C, 64·8; H, 8·2%). A mixed m.p. with the hydrogenation product of III showed no depression.

Reduction of griesenin with $NaBH_4$. Sodium borohydride (75 mg) in MeOH (5 ml) was added to a soln

of gnesenin (150 mg) in MeOH (75 ml) and left for ½ hr at room temp. The reaction mixture was then added to water (75 ml), acidified with dilute HCl and extracted with ether. The ether extract was washed with water, dried over Na_2SO_4 and removed *in vacuo* to give a residue which crystallized from acetone–ether as colourless *crystals*, VI, m.p. 169–171°, $[\alpha]_D$ +272° (*c* 0·32), λ_{max} 240 mμ (ε 14,950), ν_{max} 1769, 1638, 1605, 950, 866 and 846 cm^{-1}. (Found: C, 69·0; H, 6·9. $C_{15}H_{18}O_4$ requires: C, 68·7; H, 6·9%).

Reduction of dihydrogriesenin with $NaBH_4$. Treatment of dihydrogriesenin II (30 mg) with $NaBH_4$ (30 mg) in MeOH (5 ml) as above, gave colourless *needles*, VII, from $CHCl_3$–ether, m.p. 189°, $[\alpha]_D$ +100° (*c* 0·4), ν_{max}^{KBr} 1755 (sat. γ-lactone), 1385, 1185, 1015 and 870 cm^{-1}. (Found: M (mass spect.) 264. $C_{15}H_{20}O_4$ requires: M, 264).

Hydrogenation of VI *at* 60° *under pressure*. Compound VI (200 mg) in EtOAc was hydrogenated at 60° and 8 atm in the presence of Pt catalyst (200 mg) for 5 hr. The catalyst was filtered off, the EtOAc removed *in vacuo* and the residue (180 mg) chromatographed on silica gel (20 g).

Benzene–$CHCl_3$ (1:1) eluted fractions which after combination and evaporation of the solvent, gave a residue (140 mg) which crystallized from acetone–ether as needles of VIII, m.p. 161·164°, $[\alpha]_D$ +11° (*c* 0·58), ν_{max} 1768, 1390, 1124 and 974 cm^{-1}. (Found: C, 67·5; H, 8·2. $C_{15}H_{22}O_4$ requires: C, 67·6; H, 8·3%).

Subsequent elution with $CHCl_3$ gave the crystalline *alcohol* IXa (25 mg) from $CHCl_3$–ether, m.p. 157°, $[\alpha]_D$ +45° (*c* 0·35), ν_{max} 3500 (hydroxyl), 1760 (sat. γ-lactone) and 1080 cm^{-1}. (Found: M (mass spect.) 237 ($M-CH_2OH$). $C_{15}H_{24}O_4$ requires: M, 268).

Acetylation of IXa with pyridine–Ac_2O gave the crystalline *acetate* IXb from $CHCl_3$–ether, m.p. 137°, $[\alpha]_D$ +24° (*c* 0·43), ν_{max}^{KBr} 1755 (sat. γ-lactone), 1740 and 1240 cm^{-1} (acetate). (Found: C, 65·5, H, 8·6, M (mass spect.) 237 ($M-CH_2OAc$). $C_{17}H_{26}O_5$ requires: C, 65·8; H, 8·4%; M, 310).

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