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EXTRACTIVES OF *GEIJERA PARVIFLORA**

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Abstract—7-Geranyloxycoumarin, marmin, 6′-dehydromarmin, geiparvarin, 2′,3′-dihydrogeiparvarin and flindersine have been found in the extracts of the fruit of *Geijera parviflora* Lindl. (Rutaceae). The acetone ketal of marmin was also obtained and is regarded as an artifact of the isolation procedure. Peracid oxidation of 7-geranyloxycoumarin gave exclusively its 6′,7′-epoxide.

INTRODUCTION

THE PLANT family Rutaceae contains a series of degraded C_{26} triterpenes, the limonoids.[1] These compounds are believed to arise biogenetically from a tetracyclic triterpene of the euphol type through a series of degradations and oxidations.[1, 2] The latter stages of the metabolism can be inferred from the structures of limonoids occurring in various plants of the Rutaceae and Meliaceae.[1] A good correlation exists between the oxidation level, especially at C-19, and the systematic distribution of limonoids in the major subfamilies of the Rutaceae.[3] This leads to the question—can a similar distribution of oxidation levels be found in other classes of metabolites occurring in the Rutaceae?

Many coumarins and alkaloids occurring in the Rutaceae have isopentenyl and geranyl side chains that are often found extensively modified in the plant. Typically, such side chains are found epoxidized, hydroxylated or ring closed through oxidative cyclization[4] leading eventually to furocoumarins[5] and furoquinoline alkaloids.[6] Cases are also known in which catabolism of terpenoid side chains depart from these usual patterns, for example, in bruceol,[7] micromelumin[8] and geiparvarin.[9,10] More precisely, can the oxidation levels of such terpenoid isopentenyl and geranyl side chains be correlated with their taxonomic

* Part VIII in the series "Chemotoxonomy of the Rutaceae". For Part VII see D. L. DREYER, *J. Org. Chem.* **35**, 2420 (1970).

[1] D. L. DREYER, *Forschr. Chem. Org. Naturstoffe* **26**, 190 (1968); G. P. MOSS, *Planta Med.* Suppl. 86 (1966).
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[9] F. N. LAHEY and J. K. MACLEOD, *Austral. J. Chem.* **20**, 1943 (1967).
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distribution in the same way as the oxidation levels of limonoids correlate with their taxonomic distribution?

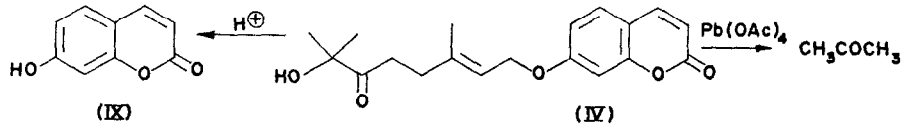
This paper describes work on such extractives occurring in the seeds of *Geijera parviflora* Lindl. Lahey *et al.*[9, 10] have recently reported the isolation and structure determination of geiparvarin (VI) from the leaves of this plant. In the present study, chromatography of acetone seed extracts yielded seven compounds. Six of these were 7-geranyloxycoumarin derivatives (I, III–VII) and the seventh was the known 2-quinolone alkaloid, flindersine (VIII).

RESULTS

The first compound eluted from the column was 7-geranyloxycoumarin (I), which has been found in a number of rutaceous plants, *Citrus paradisi* Macf.,[11] *Ptelea crenulata* Greene,[12] *Dictamnus albus* L.[13] *Aegle marmelos* Correa[14] and *Feronia elephantum.*[15]

Further elution of the column gave marmin (III),[16,17] previously found in *Aegle marmelos* and *Citrus paradisi.* Later fractions gave geiparvarin (VI)[9,10] and finally flindersine (VIII).[18,19]

The combined residues from these operations were rechromatographed and by fractional crystallization, three further coumarins were obtained. The first, m.p. 76·5–78°, analyzed for $C_{19}H_{22}O_5$, was relatively non-polar and showed the typical blue fluorescence on TLC and UV spectrum of a 7-alkoxycoumarin. The IR spectrum was similar to that of marmin (III) and 7-geranyloxycoumarin (I) and showed a hydroxy band at 3440 cm^{-1} and a carbonyl band at 1705 cm^{-1}. The presence of a keto group was confirmed by the formation of a semicarbazone and *p*-nitrophenylhydrazone. The NMR spectrum showed three *C*-methyl resonances. Two of these were displaced slightly downfield and correspond to *C*-methyls on a carbon attached to a single-bond oxygen function. A three-proton broadened resonance at δ 1·82 was consistent with the presence of a vinyl *C*-methyl group. Further, a vinyl triplet coupled with an allyl ether methylene as well as an A_2B_2 system in the region δ 2–3 could be easily distinguished. The position of a hydroxy resonance at about δ 4·5 was temperature dependent and disappeared upon addition of D_2O. These data are consistent with structure IV. The presence of the α-hydroxyketone group was shown by cleavage with lead tetraacetate to give acetone. The presence of an allyl ether group was shown by mild acid hydrolysis yielding umbelliferone (IX). Finally, reduction of 4 with KBH_4 gave racemic marmin (III).



(IX) (IV)

[11] W. L. STANLEY and S. H. VANNIER, *Phytochem.* **6**, 585 (1967).
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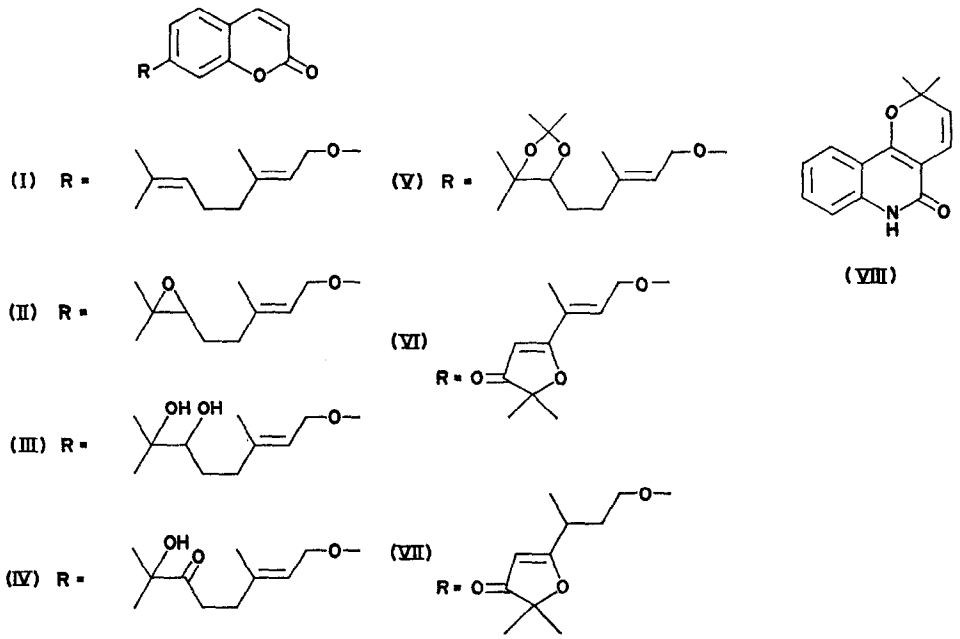
[18] R. F. C. BROWN, J. J. HOBBS, G. K. HUGHES and E. RITCHIE, *Austral. J. Chem.* **8**, 348 (1954); R. F. C. BROWN, G. K. HUGHES and E. RITCHIE, *Chem. & Ind.* 1385 (1955).
[19] D. LAVIE, N. DANIELI, R. WEITMAN and E. GLOTTER, *Tetrahedron* **24**, 3011 (1968).

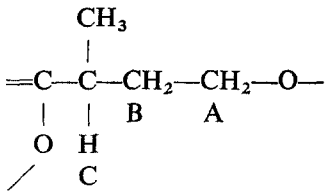
The second new product, m.p. 79–80°, showed the typical 7-alkoxycoumarin UV chromophore and blue fluorescence in UV light. Its NMR spectrum was very similar to that of marmin (III) except for the presence of two additional *C*-methyl resonances. The IR spectrum was completely free of hydroxy adsorption. These data are consistent with its formulation as the acetone ketal of marmin (V). Compound V is regarded as an artifact formed during extraction of the plant material with acetone. It is not clear, however, if the acetonide (V) is formed directly from marmin (III) or from the 6′,7′-epoxide (II)[20] by acid catalysis.



The third new product, m.p. 123·5–124·5° was optically active and had a blue fluorescence in UV light on TLC. The UV spectrum was not that of a simple 7-alkoxycoumarin. The IR spectrum suggested the presence of two different carbonyl groups in the system, ν 1715, 1675 cm^{-1}. The NMR spectrum in $CDCl_3$ had some of the aliphatic resonances overlapped so that the splitting pattern was not clearly discernable. However, the aromatic region clearly indicated the presence of a 7-alkoxycoumarin system. The NMR spectrum in benzene showed all the aliphatic resonances clearly separated from one another. The NMR spectrum showed two *C*-methyl singlets, similar to those in geiparvarin (VI) as well as an upfield *C*-methyl doublet. A sharp vinyl singlet at δ 5·33 compared well with a similar singlet at δ 5·73 in the spectrum of geiparvarin (VI) which was assigned to H-5. The chemical shifts and multiplicities of the remaining aliphatic resonances were consistent with the part structure (a).

A two-proton triplet at δ 4·04 was assigned to protons A. A two-proton multiplet at δ 2·10 corresponded to the methylene group B in a saturated environment. A one-proton four line pattern at δ 2·96 was assignable to an allyl proton *C* which was further coupled to a *C*-methyl group. These assignments were confirmed by NMR spin decoupling experiments. Irradiation of the *C*-methyl doublet collapsed the one proton quartet to a triplet

[20] M. T. Bogert and R. L. Roblin, *J. Am. Chem. Soc.* **55**, 3741 (1933).



(a)

while irradiation of the quartet collapsed the *C*-methyl doublet to a singlet. Irradiation of the multiplet at δ 2·10 collapsed the two-proton triplet at δ 4·04 to a singlet.

These data are consistent with structure VII. Compound VII, 2′,3′-dihydrogeiparvarin, has previously been prepared by the reduction of geiparvarin (VI) and the physical constants found corresponded well with those reported.[9]

In an attempt to synthesize marmin (III), 7-geranyloxycoumarin (I) was carefully epoxidized with 1 mole of *m*-chloroperbenzoic acid. Selective epoxidation of the terminal double bond occurred to give the epoxide (II). Repeated attempts to effect oxalic acid catalyzed hydrolysis of the oxide (II) to marmin (III) under a variety of conditions was unsuccessful. Hydrolysis of the allyl ether group in II occurred instead, giving umbelliferone (IX).[21]

The oxide (II) is a reasonable intermediate in the biosynthesis of marmin (III). With a sample of the synthetic oxide (II) as a reference, the mother liquors remaining from the extracts were screened for the natural oxide by TLC. A blue fluorescing spot corresponding in R_f to the synthetic oxide was present.

The coumarins found in this study can be arranged in a reasonable biogenetic sequence of increasing oxidation level. Two important points stand out, however. Firstly, the fruit of *G. parviflora* shows remarkable ability for accumulating the intermediates in the biogenetic sequence leading to geiparvarin. Secondly, the natural occurrence of dihydrogeiparvarin (VII) is quite remarkable. Isopentenyloxy and geranyloxy groups are widespread among the extractives of the Rutaceae[22] while the corresponding dihydro derivatives are almost nonexistent. Extractives of the Rutaceae are characterized by their high oxidation levels and seldom show evidence for having undergone biogenetic reductive processes.

EXPERIMENTAL

NMR spectra were taken at 60 MHz and are given in δ relative to internal TMS. The relative areas of the peaks were consistent with their assignment. Coupling constants are given in Hz.

Isolation. Fruit of *G. parviflora* was collected from two trees growing on the campus of the University of California at Riverside. Fruit from two different seasons was studied and substantially the same results were obtained. Solvent was removed from the acetone extracts of the ground fruit and the residue chromatographed on acid washed alumina. Fractions from the column were monitored by silicica acid TLC using a benzene–EtOAc (1:1). The initial hexane eluents did not show any fluorescence on TLC. They appeared to contain only terpenes and were discarded. The first fractions showing fluorescence were worked up to give compound (I), m.p. 59–60·5°, from hexane. The IR spectrum was identical with that of samples isolated from *Ptelea crenulata* and *Citrus paradisi*. Workup of subsequent hexane eluents gave V, m.p. 79–80°, after recrystallization from hexane; ν 1731, 1618 cm^{-1}; λ_{max}^{EtOH} ~243, ~253, ~300, 324 nm; NMR δ 7·58 (d, J = 9·5) H-4, 7·38 (d, J = 9) H-5, 6·60–6·85 (m) H-6 and H-8, 6·15 (d, J = 9·5) H-3, 5·45 (t, J = 6) H-2′,

[21] The oxide (II) has been found in *Aster* sp. (F. BOHLMANN, C. ZDERO and H. KAPTEYN, *Ann.* **717**, 186 (1968)); and converted to marmin; see also, R. M. COATES and L. S. MELVIN, JR., *Tetrahedron* **26**, 5699 (1970).

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4·56 (d, $J = 6$) H-1′, 3·60 (m) H-6′, 1·80–1·30 (m) H-5′, 1·78 vinyl *C*-methyl, 1·40, 1·30, 1·23, 1·19 *C*-methyls ($CDCl_3$).

Fractions eluted with 10–30% benzene in hexane contained much β-sitosterol. Solvent was removed from these fractions and the residue taken up in methanol. Cooling gave several crops of β-sitosterol. Methanol was removed from the mother liquors and the residue crystallized from EtOAc–hexane to give 2′,3′-dihydrogeiparvarin (VII), m.p. 123·5–124·5°, from EtOAc–hexane; $[\alpha]_D = 13°$ (EtOH); ν 1717, 1675 cm^{-1}; λ_{max}^{EtOH} ~243, 254, 263, ~297, 322 nm; NMR δ 7·58 (d, $J = 9·5$) H-4, 7·32 (q, $J = 9$, $J = 1$) H-5, 6·68–6·84 (m) H-6 and H-8, 6·17 (d, $J = 9·5$) H-3, 5·33 (s) H-5′, 4·04 (t, $J = 6$) H-1′, 2·96 (q, $J = 6·8$) H-3′, 2·10 (m) H-2′, 1·38 (s) 7′-*C*-methyls, 1·33 (d, $J = 6$) 3′-*C*-methyl ($CDCl_3$); 5·92 (d, $J = 9$) H-3, 5·26 (s) H-5′, 3·62 (t, $J = 6$) H-1′, 4·26 (q, $J = 7$) H-3′, 1·73 (pent, $J = 7$) H-2′, 1·25 (s) *C*-methyls, 0·95 (d, $J = 7$) *C*-methyl(benzene). The IR spectrum was identical in all respects with that of an authentic sample provided by Dr. J. K. MacLeod.

Workup of fractions eluted with 30–50% benzene in hexane gave geiparvarin (VI), m.p. 160–161·5°, from EtOAc–hexane; λ_{max}^{EtOH} 215, 236, ~300, 313 nm; NMR δ 7·85 (d, $J = 9·5$) H-4, 7·59 (d, $J = 8·3$, $J = 1·3$) H-5, 7·01 (q, $J = 7$, $J = 2$) H-6 and H-8, 6·90 (t, $J = 6$) H-2′, 6·35 (d, $J = 9·5$) H-3, 5·73 (s) H-5′, 4·95 (d, $J = 6·5$) H-1′, 2·07 vinyl *C*-methyl, 1·43 *C*-methyls ($CDCl_3$). The IR spectrum was identical in all respects with that of an authentic sample provided by Dr. J. K. MacLeod.

Further workup of the mother liquors gave dehydromarmin (IV), m.p. 76·5–78°, from EtOAc–hexane; ν 3440, 1705, 1612 cm^{-1} (Nujol); λ_{max}^{EtOH} ~243 (3·70), ~257 (3·57), ~300, 323 (4·22) nm; NMR δ 7·56 (d, $J = 9·5$) H-4, 7·44 (d, $J = 9$, $J = 1$) H-5, 6·96–6·73 (m) H-6 and H-8, 6·25 (d, $J = 9·5$) H-3, 5·53 (t, $J = 6·5$) H-2′, 4·61 (d, $J = 6·5$) H-1′, 3·91 (broad singlet) hydroxy, 2·86 (t, $J = 7$) H-5′, 2.42 (t, $J = 7$) H-4′, 1·82 (s) vinyl *C*-methyl, 1·42 (s) *C*-methyls ($CDCl_3$). Found: C, 68·9, H, 6·75. Calc. for $C_{19}H_{22}O_5$: C, 69·07; H, 6·71%.

Dehydromarmin semicarbazone, m.p. 179–180°, from MeOH. Found: C, 62·2; H, 6·46; N, 10·5, Calc. for $C_{20}H_{25}N_3O_5$; C, 61·9, H, 6·50, N, 10·8%.

Dehydromarmin p-*nitrophenylhydrazone*, m.p. 125–126°, from MeOH. Found: C, 64·1: H, 5·8. Calc. for $C_{25}H_{27}N_3O_6$: C, 64·50; H, 5·84%.

Fractions eluted with 50–80% benzene in hexane gave a crop of dehydromarmin (IV). Workup of the mother liquors gave flindersine (VIII), m.p. 196–199°, from EtOAc–hexane; lit.[19] m.p. 196–198°. The IR spectrum was identical in all respects with that of a sample provided by Professor E. Ritchie.

Fractions eluted with benzene were concentrated and the residue taken up in EtOAc–hexane. Marmin (III) crystallized upon cooling, m.p. 122–123·5°; ν 3480, 3410, 1720, 1700, 1618 cm^{-1}; λ_{max}^{EtOH} ~241, ~251, ~300, ~325 nm; Found: C, 68·7, H; 7·11. Calc. for $C_{19}H_{24}O_5$: C, 68·65; H, 7·28%.

The crude seed extracts failed to show any evidence for the presence of limonoids by TLC and spraying with Ehrlichs' reagent.[23]

Lead tetraacetate oxidation of 6′-dehydromarmin (IV). To a solution of 30 mg of dehydromarmin in HOAc was added 100 mg of $Pb(OAc)_4$. The solution was heated with a distilling head arranged so that the acetone as it was formed could be driven over in a stream of N_2 into a solution of 2,4-dinitrophenylhydrazine in a centrifuge tube. The 2,4-dinitrophenylhydrazone was collected and filtered through a short column of alumina with $CHCl_3$. It proved to be identical in all respects with an authentic sample of acetone 2,4-DNP.

Hydrolysis of 6′-dehydromarmin (IV). Four drops of conc. HCl were added to a solution of 6′-dehydromarmin in HOAc and the solution refluxed for 45 min. The solution was cooled, diluted with water and extracted with ether. Solvent was removed from the dried ether extracts and the residue crystallized from EtOAc to give 7-hydroxycoumarin (IX), identical with a commercial sample.

Reduction of dehydromarmin. To a solution of 200 mg of dehydromarmin in 5 ml of 95% EtOH was added an excess solution of KBH_4 in 95% EtOH. The solution was refluxed for 30 min. The solution was poured into excess water and extracted with EtOAc. Solvent was removed from the dried extracts and the residue filtered through a short column of alumina with benzene. Solvent was removed from the filtrates and the residue recrystallized 2× from EtOAc–hexane to give DL-marmin, m.p. 117–118°. The product was identical with natural marmin by TLC, IR and NMR criteria.

6′,7′-Epoxy-7-geranyloxycoumarin (II). A solution of 1 g of 7-geranyloxycoumarin and 350 mg of *m*-chloroperbenzoic acid was allowed to stand overnight in $CHCl_3$ at room temp. The solution was then washed with 5% Na_2CO_3, filtered through a short column of alumina and the solvent removed. The residue was crystallized from hexane to give, II, m.p. 52–54°; NMR δ 7·65 (d, $J = 9$) H-4, 7·40 (d, $J = 7$) H-5, 6·87 (q, $J = 7$, $J = 2$) H-6, 6·80 (d, $J = 2$) H-8, 6·23 (d, $J = 9$) H-3, 5·53 (t, $J = 7$) H-2′, 4·62 (d, $J = 6$) H-1′, 2·72 (t, $J = 6$) H-6′, 2·40–2·10 (m) H-4′, 1·78 vinyl methyl, 1·60 (m) H-5′, 1·28, 1·27 *C*-methyls ($CDCl_3$). Found: C, 73·0; H, 6·99. Calc. for $C_{19}H_{22}O_4$: C, 72·59; H, 7·05%.

Acknowledgements—The authors are indebted to Drs. E. Ritchie, J. MacLeod, A. C. Weiss and R. Molyneux for making comparison samples available and to Dr. E. Motell for aid in the decoupling experiments.

[23] D. L. DREYER, *J. Org. Chem.* **30**, 749 (1965).

Key Word Index—Geijera parviflora; Rutaceae; prenylcoumarins.