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CITRUS BITTER PRINCIPLES—X[1]

EXTRACTIVES OF *SWINGLEA GLUTINOSA* (Bl.) Merr.

D. L. DREYER

Department of Chemistry, San Francisco State College, San Francisco, California

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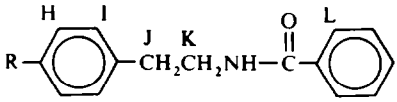
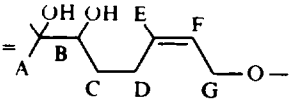
Abstract—A severine alkaloid(**3**), a C_{30} triterpene(**8**) and a related acetate (**9**) have been isolated from extracts of the fruit of *Swinglea glutinosa* (Bl.) Merr. (Rutaceae). Spectroscopic studies, including NMR spin decoupling, as well as hydrolytic experiments indicate that the alkaloid should be formulated as **3**. The synthesis of alloseverine(**7**) is described and indicates that severine alkaloids have neryl stereochemistry. The triterpene(**8**) is of the protolimonoid type and is a mixture of C-21 isomers related to melianone(**8**). Oxidation of the triterpene gave melianone lactone (3-oxo-3β-deacetylturraeanthin lactone)(**10**).

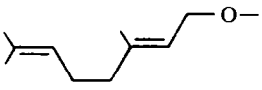
AS PART of a search for C_{26} terpenoids of the limonoid type[2] in *Citrus* relatives, the extractives of *Swinglea glutinosa* (Bl.) Merr. (Rutaceae)[3] have been examined. This species is native to the Phillipine Islands and has a very sticky, pitchy coating on the fruit. Seed extracts failed to show any evidence for fluorescence compounds or for the presence of limonoids by TLC and spraying with Ehlich's reagent.[4]

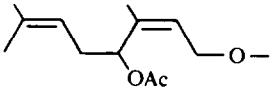
Extracts of the whole, dried fruit without the seeds gave, after chromatography on alumina, a protolimonoid, its acetate and an alkaloid which proved to be a new severine derivative.[5] The general nature of the alkaloid was suggested by (*a*) its UV spectrum, which was identical with that of severine(**1**),[5] (b) the general similarity of its IR spectrum to that of severine(**1**) and finally by (*c*) its NMR spectrum. The IR spectrum showed a carbonyl band at 1751 cm^{-1} and a sharp band at 3344 cm^{-1} which was assigned to an N—H group. The ester nature of the CO group was suggested by an intense broad band at 1250 cm^{-1}. The NMR spectrum of the alkaloid showed all the aromatic resonances of severine(**1**)[5] (Table 1). Further, resonances for three vinyl Me groups, an acetoxy Me group, two one-proton vinyl triplets, a two-proton allyl ether doublet, and a one-proton methine resonance could be readily distinguished in the NMR spectrum. The chemical shifts and multiplicities of these resonances compared well with the corresponding resonances in a synthetic sample of N-benzoyl-O-geranyltyramine(**2**) (Table 1). At 60 MHz one of the vinyl resonances was badly overlapped with the methine resonance so that their multiplicity was not easily distinguishable. These results suggest that the alkaloid is a N-benzoyl-O-geranyltyramine derivative(**2**) in which an OAc group is located at either the 4′- or 5′-positions. Partial resolution of the overlapped resonances was obtained by running the NMR spectrum in benzene in which the bands were only partly overlapped. Moreover, the overlapped resonances were fully resolved at 100 MHz and each proved to be a triplet. This multiplicity is only consistent with the OAc group at the 4′- position as in **3** and excludes location of the OAc group at the 5′- position.

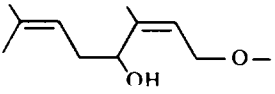
Base saponification of the alkaloid(**3**) gave the corresponding alcohol(**4**), confirming



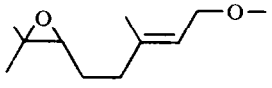
1 R = 

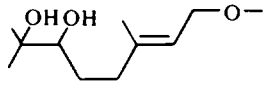
2 R = 

3. R = 

4. R = 

5. R = HO—

6. R = 

7. R = 

the presence of an OAc group. The 4′-triplet moved upfield to δ 4·01 in the NMR spectrum of the deacetyl derivative(**4**), Table 1. The triplet was not overlapped by any other bands. Spin decoupling experiments on **4** showed that irradiation of the triplet at δ 2·27 assigned to H-5′ collapsed the triplet for H-4′ (δ 4·01) into a singlet. However, irradiation of the H-4′ triplet collapsed the H-5′ triplet to a doublet, indicating that H-5′ is coupled to a further resonance, the H-6′ vinyl proton. This was shown by irradiation of the H-5′ triplet whereby the vinyl triplet at δ 5·05 collapsed to a singlet. Chemical evidence for the presence of an allyl ether group in the alkaloid(**3**) was provided by mild acid hydrolysis from which N-benzoyltyramine(**5**) was obtained. These data indicate the alkaloid is N-benzoyl-4-(4′-acetoxyneryloxy)phenethylamine(**3**). Rotational measurements of the alkaloid indicate that it is optically inactive.

The synthesis of severine was undertaken in order to provide firm evidence for the structures of this series of alkaloids. The terminal double bond of N-benzoyl-O-geranyltyramine(**2**) was selectively epoxidized with one mole of *m*-chloroperbenzoic acid in chloroform. Special solvent systems for epoxidation on a terminal double bond[6] were not necessary in this case. Attempted acid hydrolysis of the epoxide(**6**) to severine(**1**) with oxalic acid under a variety of conditions was unsuccessful. Hydrolysis of the allyl ether linkage occurred preferentially in all cases. Finally, a diol

TABLE 1. NMR SPECTRA OF SEVERINE AND DERIVATIVES IN DEUTERIOCHLOROFORM

Protons	A	B	C	D	E	F	G	H	I	J	K	L	Others
Compound													
2	1·67	5·10(t)	2·05	2·05	1·58	5·50	4·50	6·87(9)	7·10(9)	2·80(7)	3·58(7)	7·80–7·26	
1	1·27	4·27(t)	1·80	2·80	1·72	5·75	4·52	6·82(9)	7·06(9)	2·80(6)	3·56(6)	7·79–7·29	
6	1·25 1·26	2·68(t)	1·68	~2·08	1·73	5·52	4·50	6·85(9)	7·08(9)	2·83(7)	3·63(7)	7·84–7·30	
3	1·70 1·72	~5·02	2·35(6)	5·15	1·63	5·72(6)	4·53(6)	6·85(9)	7·10(9)	2·83(7)	3·63(7)	7·82–7·30	2·00 (Acetate)
4	1·72 1·72	5·05(7)	2·27(6)	4·01(7)	1·63	5·75(7)	4·51(6)	6·91(9)	7·22(9)	2·82(7)	3·62(7)	7·90–7·30	
7	1·15	3·01(m)	2·03	2·45	1·70	5·48(6)	4·45	6·76(9)	7·10(9)	2·80(6)	3·60(6)	7·73–7·30	

was obtained by hydrolysis with 7% perchloric acid at room temperature. This diol proved to be different from severine derived from natural sources by R_f on TLC, IR spectrum and differences in the NMR spectrum.

This non-identity requires that natural severine(**1**) must have different stereochemistry about the allyl ether double bond than the synthetic product. Since synthetic alloseverine was derived from gernaiol, the vinyl methyl group must be *cis* to the ether methylene[7] and severine obtained from natural sources must be derived instead from nerol.

The chemical shift of the vinyl proton in the NMR spectra of severine(**1**) and derivatives falls into two groups. As shown in Table 2, H-2′ in the naturally occurring severine derivatives falls in the range δ 5·72–5·80 while that of the synthetic derivatives as well as some coumarins containing geranyl groups falls in the range δ 5·45–5·53. Thus the naturally occurring severine palmitate[5] and the acetate(**3**) described in this paper both have the vinyl C-Me, ether methylene *trans* as in nerol. On the other hand, the values of H-2′ in 7-geranyloxycoumarin, marmin and their derivatives[8] fall in the range δ 5·45–5·53 and therefore must have the C-Me, ether methylene *cis* as in geraniol.[9]

TABLE 2. NMR RESONANCES OF H-2′ AND STEREOCHEMISTRY OF GERANYL AND NERYL DERIVATIVES

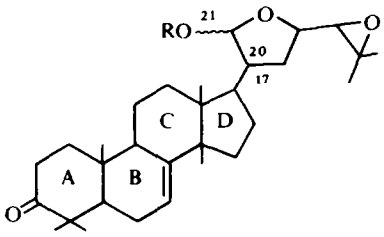
Neryl derivatives	H-2′
Severine **1**	5·75
Severine acetate[5]	5·77
Severine palmitate[5]	5·80
Severone[5]	5·79
N-Benzoyl-O-(4′-acetoxyneryl)tyramine(**3**)	5·72
N-Benzoyl-O-(4′-hydroxyneryl)tyramine(**4**)	5·75
Geranyl derivatives	**H-2′**
N-Benzoyl-O-geranyltyramine(**2**)	5·50
6′,7′-Oxide(**6**)	5·52
Alloseverine(**7**)	5·50
7-Geranyloxycoumarin[8]	5·45
Marmin[8]	5·53
Dehydromarin[8]	5·53
6′,7′-Oxide[8] of 7-Geranyloxycoumarin	5·53

Selective epoxidation of the terminal double bond of 7-geranyloxycoumarin with peracid has also been achieved.[8] Again attempted hydrolysis of the oxide group to the diol (marmin)[10] with oxalic acid gave only cleavage of the allyl ether linkage.

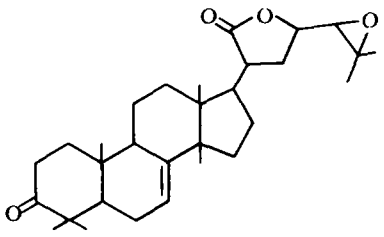
Severine occurs as the palmitate ester in *Severinia buxifolia* (Rutaceae)[5] and a severine derivative, very likely, a 4′-dehydro derivative of 2 occurs in *Casimiroa edulis* Lex *et* Llave (Rutaceae).[1]

From the more polar eluents of the column a triterpene was obtained, m.p. 205–208°, which analyzed for $C_{30}H_{46}O_4$. Its IR spectrum showed OH and CO absorption. A one-proton vinyl resonance overlapped with a resonance assigned to a single acetal proton and seven C-Me resonances could be distinguished in the NMR spectrum.

These data and the botanical origin suggested that the triterpene was possibly a C_{30} protolimonoid of which several examples, flindissol,[11] turraeanthin,[12] melianone(8),[13] and aphanamixin,[14] have been previously reported. The overlapped resonances were cleanly separated in benzene. Similar overlapping of the H-7 and H-21 resonances have been reported for melianone.[13]



8 R = H
9 R = Ac



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The relative location of the keto group to the double bond was shown by the ORD curve which was superimposible on that of other 3-keto-Δ^7-triterpenes, for example, flindissone lactone,[11] 3-oxo-3β-deacetylturraeanthin lactone,[12] lanost-7-en-3-one[15] and melianone.[13] The ORD results also establish the A/B *trans* ring juncture.

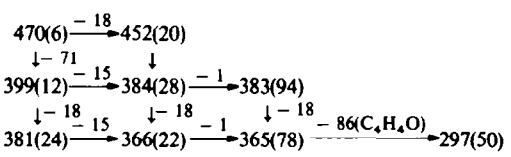
The presence of a fourth O atom in the triterpene is indicated by a molecular ion peak in the mass spectrum, *m/e* 470(6), and by the C, H analyses. Its etheral character was suggested by the absence of any indication of its functional nature in the IR. Its assignment to an epoxy group was possible by the presence of a resonance for an epoxy proton, H-24 as a pair of doublets. A similar pair of doublets has been reported for the H-24 resonance of turraeanthin (structure 8 with a 3-acetoxy group).[12] These data can be accommodated by structure 8. Chemical confirmation of structure 8 was achieved by chromic acid oxidation to give the known γ-lactone, melianone lactone(10)[13] (3-oxo-3β-deacetylturraeanthin lactone). The gross structure 8 has been previously assigned to melianone, m.p. 232–233°, an extractive of *Melia azedarach* (Meliaceae).[13] The pair of doublets for H-24 suggests that the triterepene is a mixture of C-21 isomers. One of the C-Me resonances was also a doublet (with 3 Hz spacing) again suggesting the presence of two epimers. The difference in observed physical properties of 8 from those reported[13] for melianone appears due to the presence of different ratios of the two possible C-21 isomers. Finally the IR spectrum was very similar to that of melianone, with only a few minor differences.

The non-polar eluents from the column also yielded small amounts of a triterpene acetate which appeared to be the 21-acetate of **8**. Unlike **8**, the acetate(**9**) appeared to be a single stereoisomer since H-24 appeared as a doublet in the NMR. Acetylation of 8 gave a non-crystalline acetate. The difference in physical properties must again be due to different stereochemistry at C-21. A similar non-crystalline acetate has been reported from melianone.[13]

The mass spectrum cracking pattern of **8** was generally consistent with the proposed structure and showed many similarities to those reported for turraeanthin and its 3-keto derivative.[12] The loss of water (M-18), Me (M-15) and the four C atoms of the side chain could be observed and have analogies in the cracking pattern of turraeanthin.[12] The most obvious mass spectrum fragmentation routes are outlined in

scheme 1. Relatively intense peaks at *m/e* 271(64) and 245(24) were present and are generally observed in the mass spectra of 3-keto-Δ^7-triterpenes.[16]

SCHEME 1. Mass spectral fragmentation of the triterpene(**8**)



Three protolimonoids, turraeanthin,[12] melianone(**8**)[13] and aphanamixin,[14] have previously been reported to occur in plants of the Meliaceae. However, only flindissol has heretofore been found in the Rutaceae. Moreover, it occurs only in the one of the minor subfamilies, the Flindersioideae.[17] This study is thus, the first report of protolimonoid from one of the major subfamilies of the Rutaceae, the Aurantioideae.

EXPERIMENTAL*

Isolation. Plant material was collected at the U.S. Plant Introduction Station, Coral Gables, Florida. The fruit was sliced open, the seeds removed and the sliced fruit allowed to air dry. The seed extracts did not contain any fluorescing materials but appeared to consist largely of fats. The dried, ground fruit was exhaustively extracted with acetone. Solvent was removed from the extracts and the residue chromatographed on alumina. Workup of the benzene eluents gave **3**, m.p. 64–65°, from EtOAc-hexane. Compound **3** was very soluble in MeOH; ν 3344 (N—H), 1751 (acetate), 1649 (amide), 1542 (aromatic), 1250 (ester) cm^{-1} (Nujol); λ_{max}^{EtOH} 226, ~ 272, 284 mμ. (Found: C, 74·2; H, 7·45; N, 3·26. $C_{27}H_{33}NO_4$ requires: C, 74·45; H, 7·63; N, 3·21%).

Workup of the chloroform eluents gave **8**, m.p. 205–208°, recrystallized 2X from EtOAc-hexane. The product gave a positive 2,4-dinitrophenylhydrazone test; ν 3462 (hydroxy), 1728 (keto) cm^{-1} (Nujol); NMR δ 5·35 (broad s) H-7 and H-21, 2·90 (d) J = 8 and 2·72 (d) J = 8 H-24, 1·32, 1·32, 1·13, 1·05, 1·05, 1·03, C-Me's, 0·90, 0·85 (spacing of 3 Hz) C-Me ($CDCl_3$); δ 5·42 H-21, 5·28 vinyl, 2·95 (d) J = 8 and 2·72 (d) J = 8 H-24, 1·18, 1·18, 1·03, 1·03, 0·95, 0·87, C-Me's (*benzene*); ORD in dioxan (*c*, 0·139): $[\alpha]_{600}$ −65°. $[\alpha]_{317}$ − 470°, $[\alpha]_{308}$ − 390° (sh), $[\alpha]_{277}$ + 115°, $[\alpha]_{250}$ − 50° (last reading); CD in dioxan (*c*, 0·003): 334 (0), 312i (− 0·38), 300 (− 0·61), 295i (− 0·59), 259 (0); (Found: C, 76·3; H, 9·81. $C_{30}H_{46}O_4$ requires: C, 76·55; H, 9·85%). The mother liquors from the above operations were combined, solvent removed and the residue rechromatographed on alumina. The fractions were monitored by removal of solvent and examination of the oily residue by IR. The IR spectra of the first hexane eluents suggested the presence of only fats. Later hexane fractions showed a more complex IR spectra. These were combined, concentrated, cooled and induced to crystallize by scratching, m.p. 155–160°, from CCl_4-hexane to give **9**. Compound **9** gave a positive 2,4-dinitrophenylhydrazone test; ν 1750 (acetate), 1722 (keto) cm^{-1} (Nujol): NMR δ 6·11 J = 1 H-21, 5·30, H-7, 2·57 J = 8 H-24, 2·17 acetate, 1·28, 1·27, 1·27, 1·10, 1·00, 0·87, C-Me's, ($CDCl_3$).

Melianone lactone (**10**). The triterpene **8** was oxidized with chromic acid in pyridine. After workup the product was filtered through a short column of alumina and after removal of solvent the residue was crystallized from MeOH-water. The product was identical in all respects with a sample of melianone lactone provided by Professor Lavie.

Saponification of **3**. A solution of 40 mg of **3** in methanolic KOH aq was refluxed for 30 min. The mixture was then poured into water and extracted with chloroform. The chloroform extracts were dried, solvent removed and the residue crystallized from EtOAc-hexane to give **4**, m.p. 117–118°; ν 3330 (N—H and O—H), 1643 (amide) cm^{-1} (Nujol). (Found: C, 76·0; H, 7·91. $C_{25}H_{31}NO_3$ requires: C, 76·30; H, 7·94%).

Hydrolysis of N-*benzoyl*-4-(4′-*acetoxygeranyloxy*)*phenethylamine*(**3**). A soln of 30 mg of **3** in 3 ml AcOH

* NMR spectra were taken at 60 MHz. The relative areas of the peaks were consistent with their assignments.

and 2 drops conc HCl were refluxed for 30 min. Workup of the reaction mixture gave **5**, identical in all respects with a synthetic sample.[19]

N-Benzoyl-4-geranyloxyphenethyl amine (**2**). An acetone soln of geranyl bromide[20] (5 g) and N-benzoyltyramine[19] (5 g) was refluxed 20 hr over anhyd K_2CO_3. The soln was filtered and concentrated. The product (**2**) crystallized upon addition of MeOH and cooling, m.p. 101·5–103°, after recrystallization from MeOH; 6 g yield; ν 3365 (N—H), 1645 (amide), 1563, 1528 cm^{-1} (Nujol). (Found: C, 79·5; H, 8·17; N, 3·78. $C_{25}H_{31}NO_2$ requires: C, 79·53; H, 8·27; N, 3·95%).

Epoxidation of **2**. A soln of 300 mg of **2** and 100 mg of *m*-chloroperbenzoic acid was allowed to stand overnight in chloroform at room temp. The soln was then washed with 5% Na_2CO_3 aq, filtered through a short column of alumina and the solvent removed. The residue was scratched with a little cold hexane, whereupon **6** crystallized, m.p. 77–78° after recrystallization from EtOAc-hexane; ν 3358 (N—H), 1642 (amide), 1587, 1548 cm^{-1} (Nujol). (Found: C, 76·7; H, 7·89; N, 3·56. $C_{25}H_{31}NO_3$ requires: C, 76·30; H, 7·94; N, 3·56%).

The use of excess *m*-chloroperbenzoic acid gave the dioxide; m.p. 101–102°, from EtOAc-hexane; (Found: C, 73·5; H, 7·47. $C_{25}H_{31}NO_4$ requires: C, 73·34; H, 7·63%).

Alloseverine(**7**). To a soln of **6** (50 mg) in dioxan (8 ml) was added 7% $HClO_4$ (4 ml). The soln was allowed to stand 1·5 hr at room temp. The mixture was decomposed with water and extracted with chloroform. The chloroform extracts were dried and solvent removed. The residue was chromatographed on a short column of alumina. The product was eluted with chloroform, m.p. 144–144·5°, from EtOAc-hexane. (Found: C, 72·6; H, 8·19; N, 3·12. $C_{25}H_{33}NO_4$ requires: C, 72·9; H, 8·08; N, 3·42%).

Acknowledgements—Initial experiments on this problem were carried out in the Pasadena laboratories of the U.S. Department of Agriculture. The author is indebted to Professor D. Lavie for comparison samples of melianone and melianone lactone and to Dr. E. Motell for the decoupling results.

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