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CHEMOTAXONOMY OF THE RUTACEAE—XII[a]

THE OCCURRENCE OF SEVERINE IN *ATALANTIA MONOPHYLLA* AND *HESPERETHUSA CRENULATA*. A REVISED STRUCTURE FOR SEVERINE

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Abstract—Severine palmitate has been isolated from the fruit of *Atalantia monophylla* and *Hesperethusa crenulata* both of the family Rutaceae. NMR shift reagent and mass spectral studies suggest the need for revision of the structure of severine to **2**. The revised structure is confirmed by the ^{13}C NMR spectra of severine and its derivatives.

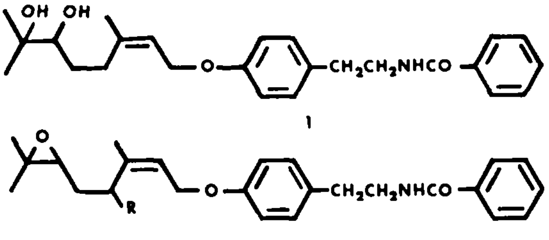
A previous paper reported[1] structure **1** for severine, a metabolite occurring as the palmitate ester in several citrus relatives.[1,2] This extractive has now been isolated from the fruit of two further citrus relatives, *Atalantia monophylla*[3] and *Hesperethusa crenulata*.[4] During this isolation work several lines of evidence suggested that the structure assigned to severine may require revision. These include, a consideration of the MS fragmentation patterns and a study of NMR shift reagents on severine and its derivatives. These indications have now been confirmed by the ^{13}C NMR spectra of severine and its derivatives. These results indicate that the structure of severine should be revised to **2**.

Previous chemical work[1] on severine showed that it was a benzamide of a tyramine ether. Ease of acid catalyzed hydrolysis showed that severine contained an allyl ether group. Oxidation of severine with Jones reagent gave a ketone, severone, indicating the presence of a secondary alcohol group. Reaction of severine with lead tetraacetate yielded acetone, isolated as its 2,4-D derivative, indicating the presence of a 1,2-diol group. These chemical data, along with spectroscopic considerations caused severine to be assigned structure **1**.[1] It now appears that the lead tetraacetate oxidation results were missleading and that instead of a 1,2-diol group the corresponding epoxide was present. The epoxide group was apparently opened up under the reaction conditions to give the corresponding 1,2-diol which was subsequently cleaved by the lead tetracetate.

The main features of the proton NMR spectra of severine and its derivatives were those signals assigned to the aromatic protons, one vinyl C-Me, two C-Me's in a saturated system and an allyl ether group. In the vinyl region were two one-proton triplets assigned to the vinyl proton of the allyl ether group and the carbinol proton of the secondary alcohol group.

Titration of severine and its derivatives with increasing amounts of Eu(fod)$_3$ resulted in downfield shifts to various degrees of many of the signals. This downfield shift of signals resulted in the uncovering of a previously unrecognized one-proton triplet centered at 2.67 ppm in severine. This triplet occurred at 2.73 ppm in the palmitate ester. With increasing aliquates of the shift reagent this triplet moved downfield more slowly in the ester than in the alcohol, severine. The occurrence of this one-proton triplet suggested the presence of additional functionality in severine and its chemical shift was consistent with the presence of an epoxide group.[6] Its triplet nature indicated the presence of two adjacent protons. These spectral features support the presence of a 6',7'-epoxide group and a 4'-OH group on a geranyloxy system.

The mass spectra of severine and its derivatives indicated that the original emperical formula proposed was two mass units too high. In spite of the presence of an allyl ether group it has now been possible to obtain molecular ion peaks for several severine derivatives supporting the revised emperical formula. In each case peaks were present which supported the presence of the 4'-hydroxy-6',7'-epoxygeranyl system. Diagonistic peaks supporting the revised structure in the case of severine



1

2 R=OH

4 R=H

5 R=OAc

6 R=$-OCO(CH_2)_{14}CH_3$

Fig. 1.

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[a] For part XI see D. L. Dreyer and K. H. Park, *Phytochemistry* 14, 1617 (1975).

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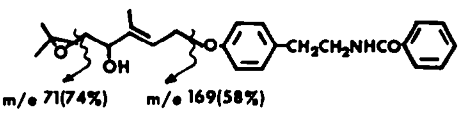

Fig. 2.

are *m/e* 71 and 169 as well as a molecular ion peak at *m/e* 409. Similar fragmentation patterns were followed by other severine derivatives (Experimental).

The ^{13}C NMR spectra of severine and its derivatives gave strong support for the presence of four carbons singly bonded to oxygen on the geranyl side chain. ^{13}C NMR assignments were made by comparison with literature correlation tables and internal consistency. Comparison of the ^{13}C NMR spectrum of the previously reported[5] synthetic benzamide of 0-geranyltyramine (3) with that reported[7] for geraniol allowed assignments to the carbons of the side chain to be made. The assignment of the aromatic signals in the N-benzoyltyramine fragment was made from readily available data.[8] The assignment of the adjacent methylene carbons in the N-benzoyltyramine moeity was not unambigous. The signals for these carbons came at 39.1 and 63.8 ppm in 2-phenylethanol; at 40.2 and 43.7 ppm in 2-phenyl-ethylamine; at 39.8 and 43.7 ppm in 3,4-dimethoxy-β-phenethylamine;[9] and at 41.9 and 36.4 ppm in N-benzoyl-2-phenylethylamine. On the assumption that the position of the benzylic carbon would remain relatively constant in this series of compounds, the signals at 41–42 ppm are tentatively assigned to the benzylic carbon and those at 35 ppm assigned to the amino carbon in the severine derivatives.

Comparison of the carbon NMR spectrum of the geranyloxy derivative (3) with that of the related epoxide (4)[5] permitted assignments of the 6'- and 7'-carbons. Finally comparison of the spectrum of the synthetic epoxide (4) and acetate ester (7) with that of severine, severine acetate (5) and palmitate (6) allowed complete assignments to be made of the naturally occurring series.

The assignment of signals for the palmitate carbons of 6 was made with the aid of data from methyl myristate.[10] These ^{13}C NMR data compel a revision of the structure for severine from structure 1 to 2. The UV

Table 1. ^{13}C NMR data for severine and derivatives[a]

Compound	3	4	7	5	2	6[c]
Proton						
a	131.5	131.5	131.5	131.3	131.3	130.8
b	127.8	127.7	127.8	126.9	126.9	126.9
c	128.9	128.7	128.9	128.3	128.4	128.4
d	140.4	140.0	137.8	137.0	141.4	141.4
e	167.0	167.0	167.1	167.4	167.4	166.6
f	35.5	35.4	35.5	34.8	34.8	34.8
g	42.2	42.2	42.2	41.4	41.4	41.3
h	b	b	b	b	131.1	b
i	130.3	130.2	130.4	129.6	129.7	129.5
j	115.1	115.1	115.4	114.8	114.8	114.7
k	158.2	158.0	158.1	157.1	157.4	157.1
l	65.3	65.1	65.0	64.3	64.5	64.0
m	121.1	121.3	119.9	123.0	121.1	122.9
n	136.0	135.8	136.0	134.7	134.7	136.9
o	16.6	16.6	13.0	13.0	12.5	13.0
p	40.0	36.7	78.3	75.7	75.2	75.1
q	27.0	27.8	32.2	32.5	29.6	32.6
r	124.6	63.6	123.9	60.6	62.1	60.0
s	b	58.0	b	57.7	57.9	57.1
t	25.8	24.9	25.8	24.6	24.7	24.9
u	17.7	18.7	18.0	18.9	19.0	18.8
acetate Methyl			21.0	21.1		
acetate carbonyl			167.1	169.9		

a In ppm acetone d^6 solvent.

b This signal is obscured by other peaks.

c Signals of the palmitate side chain were assigned as follows: C-1, 171.7; C-2, 34.2; C-3, 24.4; C-4, to C-13, 29.6; C-14, 31.8; C-15, 22.6; C-16, 14.0 ppm.[10]

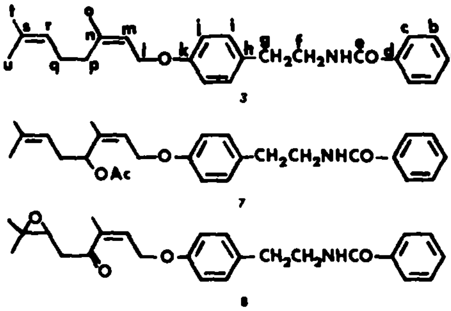

Fig. 3.

profile of severone and severine were essentially the same. The contribution of an α,β-unsaturated ketone chromophore to the UV spectrum would be swamped by the presence of the aromatic rings. Nevertheless, the presence of an α,β-unsaturated ketone system in severone was indicated by the proton NMR spectrum. A slight downfield shift of the one-proton triplet assigned to H-2′ occurred from 5.75 ppm in severine (**2**) to 5.93 ppm in severone (**8**).

Severine palmitate (**6**) has been previously reported from *Severinia buxifolia*[1] and *Pamburus missionis*.[2] Thus compound **6** has now been found in four different rutaceous species. These species are all classified in the one subfamily, Aurantioideae, of the Rutaceae.[12]

EXPERIMENTAL

^{13}C NMR spectra were taken on a JEOL PS 100 equipped with a pulsed FT system operating at 15.35 MHz. The samples were locked on the acetone D_6 solvent and all signals referenced to internal TMS.

Isolation of severine palmitate from Atalantia monophylla. Dried and ground fruit of *A monophylla* was extracted with hexane. Solvent was removed from the extracts and the concentrated extracts kept in a refrigerator overnight. A white solid appeared in the thick oil The solid was collected by filtration and washed repeatedly with cold hexane; m.p. 112–113° (benzene). The IR spectrum was super-imposible on that of a sample of **6** isolated from *Severinia buxifolia*.

Isolation of Severine palmitate from Hesperethusa crenulata. Dried and ground fruit of *H. crenulata*, obtained from the Citrus Research Center, University of California, Riverside, was extracted with benzene. Solvent was removed from the extracts and the residue chromatographed on alumina. Workup of the appropriate fractions gave 6 m.p. 113–114° from EtOAc. The IR and NMR spectra were identical with those from a sample of **6** isolated from *S. buxifolia*.

Mass spectral data **2** MS (*m*/*e* (rel. intensity) 409(6)M$^+$, 241(6) 202(6), 169(58) 122(21), 120(100), 107(23), 105(90), 83(42), 81(25), 77(57), 71(74), 55(94), 43(60).

5 MS 451(33) M$^+$, 392(27), 241(18), 240(27), 239(23), 211(24), 169(24), 152(36), 151(86), 122(29), 120(69), 109(37), 105(80), 77(56), 71(56), 55(60), 43(100).

6 MS 407(2), 241(3), 240(10), 152(17), 151(100), 139(7), 122(6), 121(5), 120(41), 109(9), 107(6), 105(30), 85(6), 81(7), 71(11), 57(11).

8 MS 407(1), 286(2), 241(2), 240(3), 168(4), 267(43), 266(8), 122(13), 121(8), 120(100), 111(6), 107(6), 105(39), 83(13), 81(8), 77(13), 69(17). 55(35), 41(7).

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