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CHEMOTAXONOMY OF THE RUTACEAE—II[1]

EXTRACTIVES OF *SEVERINIA BUXIFOLIA* (Poir.) Ten.

D. L. DREYER

Fruit and Vegetable Chemistry Laboratory[2], 263 South Chester Avenue, Pasadena, California 91106

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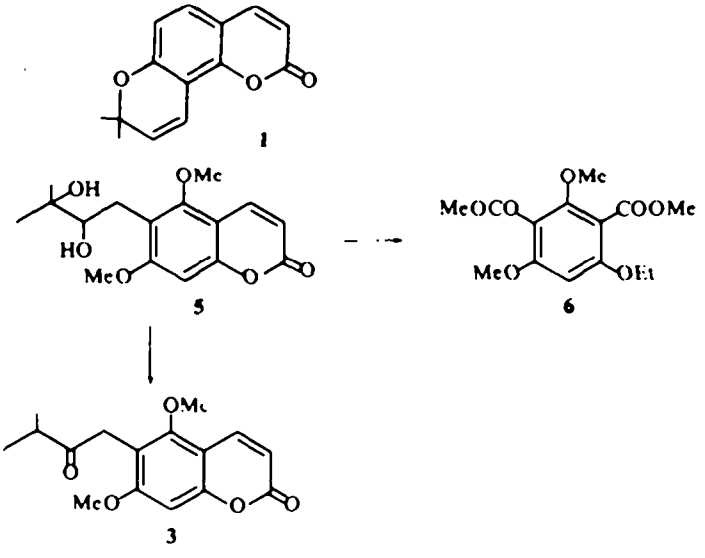
Abstract—Leaf extracts of *Severinia buxifolia* (Poir.) Ten. were found to contain seselin, small amounts of an alkaloid and a new coumarin which was shown by chemical and spectroscopic studies to be 5,7-dimethoxy-8-(3'methyl-2'-oxobutyl)coumarin. Extracts of the fruit gave small amounts of seselin, imperatorin, isopimpinelin, bergapten and the same alkaloid as that found in the leaf extracts. Base saponification of the alkaloid gave palmitic acid and an alcohol named severine. Severine is a N-benzoyl-tyramine derivative and was shown to have structure 16 by chemical and spectroscopic studies.

ALTHOUGH *Severinia buxifolia* (Poir.) Ten. and *Citrus* are both members of the same sub-family of the Rutaceae, they are not closely related botanically. However, *Citrus* species can be readily grafted onto *S. buxifolia*.[3] It is therefore of special interest to determine in a qualitative way how its chemical constituents compare with those of *Citrus*.[4]



1 5 6 3

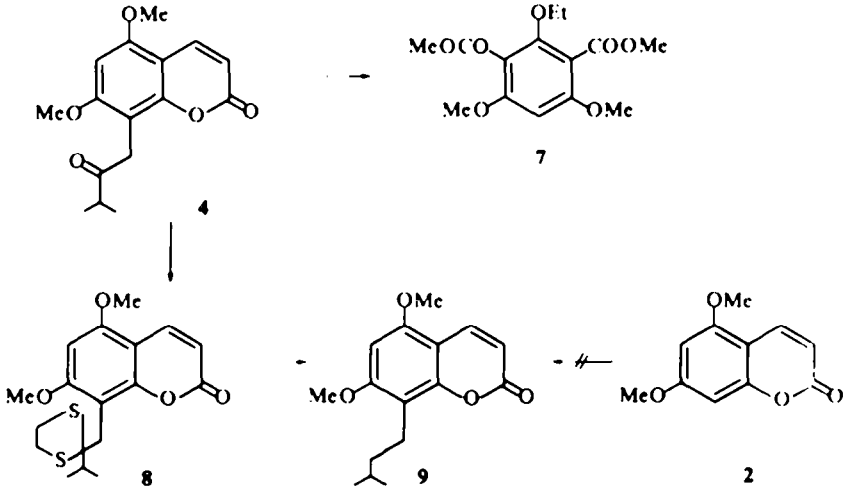
[1] Part I. D. L. Dreyer, *Phytochemistry* 5, 367 (1966).

[2] A laboratory of the Western Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture.

[3] W. T. Swingle, *The Citrus Industry* (Edited by H. H. Webber and L. D. Batchelor), Vol. I, p. 275. University of California Press, Berkeley (1943).

[4] The volatile constituents of *S. buxifolia* recently have been investigated by Scora; R. W. Scora, *Phytochem.* 5, 823 (1966).

Chromatography of leaf extracts on alumina yielded seselin (**1**), small amounts of an alkaloid which will be discussed below, and a new coumarin. This coumarin, m.p. 128–130°, analyzed for $C_{16}H_{18}O_5$ and showed a blue fluorescence, suggesting a coumarin rather than a psoralen system. The UV spectrum was similar to that of limettin (5,7-dimethoxycoumarin; **2**).[5] The IR spectrum showed an exceptionally intense carbonyl band and the coumarin readily formed a yellow 2,4-DNP and a



semi-carbazone (m.p. 234–238° dec). The NMR spectrum showed resonances for a typical coumarin AB doublet,[6] one aromatic singlet, two MeO groups and an isopropyl system.

The keto group is unconjugated with the aromatic ring since 6- or 8-acyl coumarins exhibit quite different UV spectra.[7,8] The unconjugated keto group is also consistent with the yellow rather than an orange color of the 2,4-DNP derivative and lack of change in the UV spectra of the thioketal and deoxyderivatives compared to the parent coumarin.[9] Structures **3** and **4** are consistent with these observations.

In deuteriochloroform a two proton singlet, caused by the α-methylene group, is hidden under the MeO resonances. Demonstration of the presence of this singlet was obtained from the NMR spectrum in benzene. The two methoxy resonances occurred well upfield at δ 3·35 in benzene while a two proton singlet remained at δ 3·80 compared to the spectrum obtained in deuteriochloroform.

Compound **3** is a known conversion product of toddalolactone (**5**)[10] (and aculeatin hydrate[11]) and has m.p. 120–121°; semicarbazone, m.p. 209–210°. On the basis of

[5] W. L. Stanley and S. H. Vannier, *J. Am. Chem. Soc.* **79**, 3688 (1957); H. Böhme and T. Severin, *Arch Pharm.* **290**, 486 (1957).

[6] See, for example, D. L. Dreyer, *Tetrahedron* **22**, 2923 (1966) and Ref. 1.

[7] C. Djerassi, E. J. Eisenbraun, B. Gilbert, A. J. Lemin, S. P. Marfey and M. P. Morris, *J. Am. Chem. Soc.* **80**, 3686 (1958); L. Crombie, D. E. Games and A. McCormick, *Tetrahedron Letters* 145, 151 (1966).

[8] R. A. Finnegan, B. Gilbert, E. J. Eisenbraun and C. Djerassi, *J. Org. Chem.* **25**, 2169 (1960).

[9] 5,7-Dimethoxy-8-isovalerylcoumarin, m.p. 150–151°, is a known reduction product of glabralactone; T. Kariyone and K. Hata, *J. Pharm. Soc. Japan* **76**, 649 (1956); *Chem. Abstr.* **51**, 1152 (1957).

[10] B. B. Dey and P. P. Pillay, *Arch. Pharm.* **273**, 223 (1935).

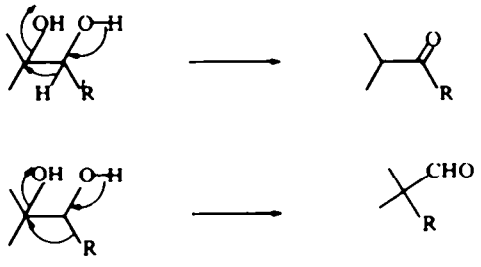
[11] P. Dutta, *J. Ind. Chem. Soc.* **19**, 425 (1942); F. M. Dean, *Naturally Occurring Oxygen Ring Compounds* p. 188. Butterworth, London (1963).

this evidence, structure **3** can be rejected for the coumarin under study leaving only **4** for consideration. More direct evidence for location of the 5-carbon side chain was obtained by a method first worked out by Späth *et al.*[12] for toddalolactone (**5**).

Exhaustive ethylation with diethyl sulfate under basic conditions, followed by oxidation with potassium permanganate, isolation of the acid fraction and esterification with diazomethane will give one of two possible diesters, **6** or **7**, depending on the orientation of the side chain. Both of these diesters have been synthesized and differ in m.p. by 35°.[12] In this manner **7** was obtained from the coumarin which is the opposite orientation to that obtained from toddalolactone (**5**). The new coumarin must be 5,7-dimethoxy-8-(3'-methyl-2'-oxobutyl)coumarin (**4**).

Further evidence for the nature of the side chain was obtained by conversion of the parent coumarin (**4**) to a thioketal (**8**). Raney Nickel desulfuration of the thioketal gave the deoxyderivative **9**, 5,7-dimethoxy-8-(3'methylbutyl)coumarin. Attempted synthesis of **9** by Friedel-Crafts reaction of limettin (**2**) with isopentyl bromide under a variety of conditions and solvents was unsuccessful. Attempted synthesis of **9** by a Pechmann reaction[13] with 2-hydroxy-4,6-dimethoxyisovalerophenone[8] and malic acid was also unsuccessful.

From the pattern of structures for extractives found in the Rutaceae it is now generally accepted that the frequently occurring isopentyl side chain can be modified by epoxidation followed by hydrolysis to a 1,2-diol.[14] This resonable hypothesis has not yet been put to the test with radio labeled materials. It now appears that the 1,2-diol can be further modified (aside from ring closure or elimination possibilities) biogenetically in two different ways formally analogous to the pinacol rearrangement. This can occur by migration of a β-proton to give a keto product as observed in the present case, or by migration of the C^{α}-C^{β} bond to give an aldehyde product of the type recently described by Fisher and Nordby.[15]



Similarly modified isopentyl side chains are found in the cases of evoxidine (**10**)[16] halfordinone (**11**),[17] and lunidonin (**12**)[18] all extractives of rutaceous plants. All of these products co-occur naturally with the corresponding 1,2-diol.

[12] E. Spath, B. B. Dey and E. Tyray, *Ber. Dtsch. Chem. Ges.* 71, 1825 (1938); *Ibid.* 72, 53 (1939).

[13] S. Sethna and R. Phadke, *Org. Reactions* Vol. VII; p. 1.

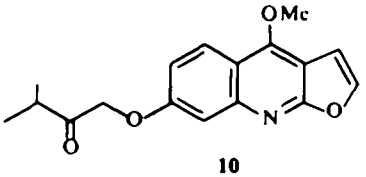
[14] W. B. Whalley in W. D. Ollis, *Recent Developments in the Chemistry of Natural Phenolic Compounds* p. 37. Pergamon, New York (1961); W. D. Ollis and I. O. Sutherland, *Ibid.* p. 83.

[15] J. F. Fisher and H. E. Nordby, *Tetrahedron* 22, 1489 (1966); W. L. Stanley, private communication; see also Ref. 14, p. 40.

[16] The case of evoxidine (**10**) may be ambiguous as it may be only an artifact; F. W. Eastwood, G. H. Hughes and E. Ritchie, *Aust. J. Chem.* 7, 87 (1954).

[17] W. D. Crow and J. H. Hodgkin, *Aust. J. Chem.* 17, 119 (1964).

[18] A. Rüegger and D. Stauffacher, *Helv. chim. Acta* 46, 2329 (1963).



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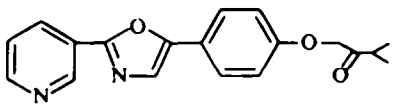

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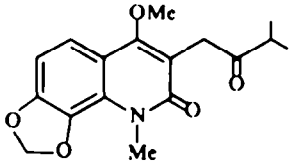

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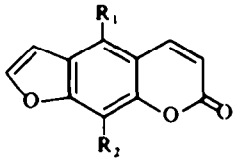
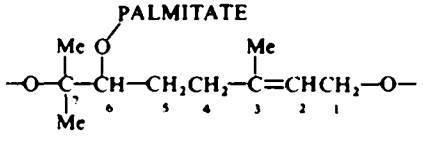


13a R_1 = H, R_2 = $-O-CH_2CH=C(CH_3)_2$

13b R_1 = MeO, R_2 = H

13c R_1 = R_2 = MeO

The fruit of *S. buxifolia* was found to contain large amounts of fats, an alkaloid, and small amounts of seselin (**1**), imperatorin (**13a**), bergapten (**13b**) and isopimpinellin (**13c**). The alkaloid was isolated on a small scale by chromatography on alumina and crystallized from benzene or ethyl acetate–hexane as a waxy product melting sharply at 113–114°. It was non-sublimable and was recovered unchanged after attempted acetylation with acetic anhydride–pyridine. The IR spectrum showed a sharp N—H or O—H band at 3330 cm^{-1} and an ester band at 1739 cm^{-1}. The natural material **19** was saponified by sodium hydroxide in aqueous dioxane to give palmitic acid and an alcohol. This alcohol, which analyzed for $C_{25}H_{33}NO_4$, has been named severine. The IR spectrum of severine (**16**) lacked the ester band but otherwise was very similar to that of the starting ester. Rotational measurements on both severine and its palmitate ester indicates that the system is optically inactive. Severine was easily converted to a monoacetate (**17**) and could be oxidized to a ketone, severone (**18**), with chromic acid. Inspection of the NMR spectra of these compounds with biogenetic considerations suggested the presence of the fragment 14.



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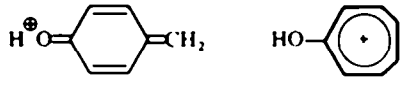
The NMR spectrum of severine (**16**) was very similar to that of the starting ester (**19**) except for changes expected by hydrolysis of a fatty acid ester, e.g. an upfield shift of a one proton symmetrical triplet showing that the natural alkaloid was the ester of a secondary alcohol (see Table 1 for summary of NMR data). The α-methylene and terminal Me groups of the fatty acid moiety present in the spectrum of **19** were lacking in the NMR spectrum of the hydrolysis product, **16**. The triplet for the secondary carbinol proton again occurred downfield in severine acetate (**17**) (Fig. 1).

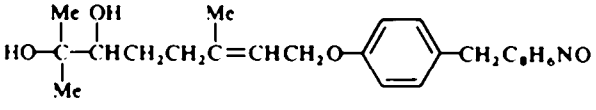
Spin decoupling of severine acetate (**17**) showed that the resonance for H-6 was coupled with a resonance at δ 1·90 indicating that H-5 was not allylic. The C-Me resonances in severine acetate occurred at δ 1·25 suggesting the presence of a single bond oxygen function at the 7-position. In addition to the vinyl methyl resonance and a two proton doublet at δ 4·54 (J = 6 c/s) coupled with a one proton olefinic triplet at δ 5·77 (J = 6 c/s) (spin decoupling),[19] the NMR spectrum showed a complex multiplet for 5-aromatic protons and resonances for an aromatic A_2B_2 system. The chemical shifts of the A_2B_2 system were strikingly similar to those of *p*-cresol methyl ether.[20]

Severine (**16**) was shown to be a 1,2-diol by reaction with lead tetraacetate. The volatile material was distilled from the reaction mixture and trapped as a 2,4-dinitrophenylhydrazone derivative. In this manner acetone 2,4-DNP was obtained. The mass spectrum of severine failed to show a molecular ion, a feature characteristic of allyl ethers.[21] The mass spectrum showed peaks consistent with the postulated C-10 side chain. Especially intense peaks were found for 6,7-bond cleavage.

The NMR and UV spectra of severine (**16**) and its derivatives suggested the presence of a *p*-alkoxylalkyl benzene moiety. The mass spectrum of **16** showed a relatively intense peak at *m/e* 107. This appears to be due to a fragment which may be either *a* or *b*. Fragmentation with proton transfer to give *a* is typical of allyl ethers, presumably via a cyclic process. The occurrence of the species *a* or *b* suggests that the allyl ether group is attached to the *p*-cresol fragment. The part structure of severine can thus be expanded to **15**.



a *b*



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Chemical proof for the presence of the allyl ether linkage was obtained by mild acid hydrolysis of severine (**16**) or **19** from which a phenolic fragment was isolated. This fragment **20** formed a monoacetate (**21**) and had the same UV chromophore as severine. The NMR spectrum of **21** showed all the aromatic resonances of severine and a two proton benzylic triplet at δ 2·84, indicating an adjacent methylene group. The resonance for the adjacent methylene group was a symmetrical quartet occurring at δ 3·58 in **21**, indicating that this resonance was also spin coupled to yet another proton. The downfield position of this resonance suggested that the second methylene

[19] For NMR data on similar geranyloxy systems in rutaceous plants see E. Ritchie, W. C. Taylor and S. T. K. Vautin, *Aust. J. Chem.* **18**, 2021 (1965); R. H. Prager and H. M. Thredgold, *Ibid.* **19**, 451 (1966).

[20] N. S. Bhacca, L. F. Johnson and J. N. Shoolery, Spectrum No. 205, NMR Spectra Catalog, Varian Associates, Palo Alto, California.

[21] See, for example, 7-geranyloxycoumarin; J. P. Kutney, G. Eigendorf, D. L. Dreyer and L. A. Mitschier, *Canad. J. Chem.* in press.

TABLE 1. NMR DATA[22] OF SEVERINE DERIVATIVES IN DEUTERIOCHLOROFORM

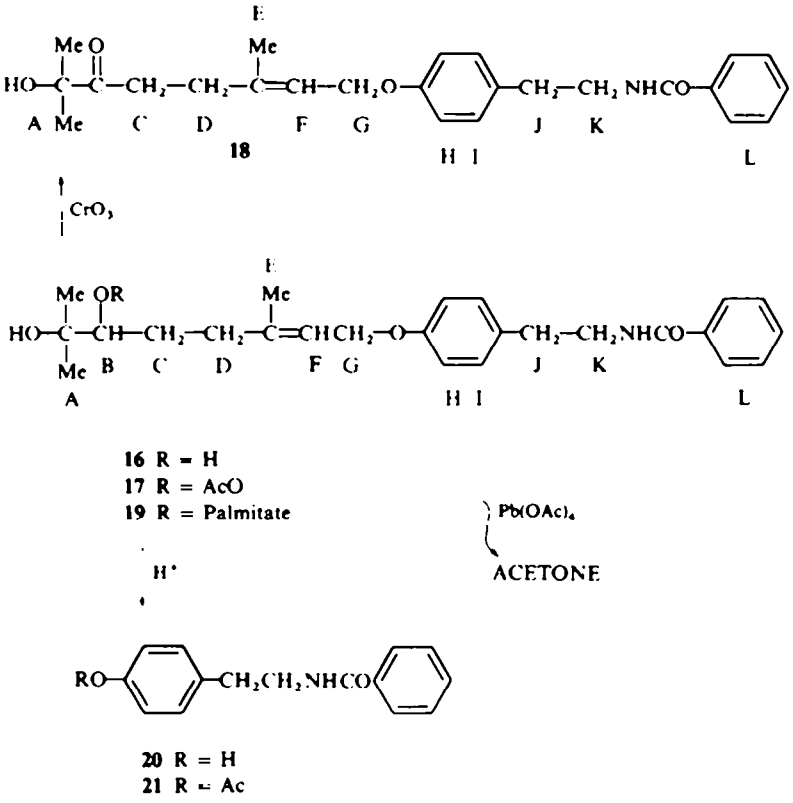
Proton	16	17	19	18
A	1·27	1·25	1·27	1·25 1·32
B	4·27(t)	5·36(t)	5·38	–
	J = 6	J = 6	J = 6	
C	1·80[a]	1·90[a]	1·90[a]	*b*
D	2·80(t)	2·70(t)	2·72(t)	*b*
	J = 6	J = 6	J = 6	
E	1·72	1·73	1·77	1·78
F	5·75(t)	5·77(t)	5·80(t)	5·79(t)
	J = 6	J = 6	J = 6	J = 6
G	4·52(d)	4·54(d)	4·57(d)	4·74(d)
	J = 6	J = 6	J = 6	J = 6
H	7·06(d)	7·07(d)	7·10(d)	7·08(d)
	J = 9	J = 9	J = 9	J = 9
I	6·82(d)	6·82(d)	6·83(d)	6·83(d)
	J = 9	J = 9	J = 9	J = 9
J	2·80(t)	2·82(t)	2·87(t)	2·89(t)
	J = 6	J = 7	J = 6	J = 6
K	3·56(t)	3·62(q)	3·60(t)	3·64(t)
	J = 6	J = 7	J = 6	J = 6
L	7·79-7·29	7·79-7·31	7·84 7·34	7·82-7·32
	(m)	(m)	(m)	(m)
Acetate		2·03		
Palmitate α-methylene			2·34(t) J = 6	
Palmitate Methylenes			1·27(s)	
Palmitate Terminal methyl			0·90(t)	

[a] Pattern partly obscured by other resonances.
[b] Pattern obscured respectively by C and D.

group was attached to either oxygen or nitrogen. Irradiation of the δ 3·58 quartet collapsed the δ 2·84 triplet into a singlet. Irradiation of the δ 2·84 triplet collapsed the δ 3·58 quartet into a doublet. The IR spectrum of **21** showed a sharp band at 3328 cm^{-1} and a pair of bands falling in the range 1640–1544 cm^{-1} for all severine derivatives which suggested the presence of a secondary amide group. The five proton aromatic multiplet in the NMR spectrum was strikingly similar to that of benzoic acid. These data suggested that the phenolic hydrolysis product is N-benzoyltyramine (**20**). The observed physical constants for **20** and **21** agreed with those published,[22] and complete identity was shown by comparison with a synthetic sample.

The evidence thus suggests that severine is the N-benzoylamide of *p*-[(6,7-dihydroxy-3,7-dimethyl-2-octenyl)oxy]β-phenethylamine (**16**). The isolation of N-Benzoyltyramine (**20**) has previously been reported from *Casimiroa edulis* Llave *et* Lex., a

[22] G. Barger, *J. Chem. Soc.* 1123 (1909); G. Barger and G. S. Walpole, *Ibid.* 1720 (1909).



member of the Rutaceae.[23, 24] The dihydroxygeranyloxy moiety present in severine is also present in a coumarin derivative recently reported from *Citrus paradisi* Macf.[25]

While the extractives found in *S. Buxifolia* are typically rutaceous,[26] they are similar to those of *Citrus* species only in their coumarin content.[27] Alkaloids are not common in *Citrus* species[28] while flavonoids[29] and limonoids[1] which are present in *Citrus* species appear to be lacking in *S. buxifolia*.

[23] F. A. Kincl, J. Romo, G. Rosenkranz and F. Sondheimer, *J. Chem. Soc.* 4163 (1956).

[24] Other tyramine derivatives isolated from rutaceous plants are O-methyltyramine-N-methylcinnamide from *Zanthoxylum americanum* Mill., F. B La Forge and W. F. Barthel, *J. Org. Chem.* **9**, 250 (1944) and aegelin from *Aegle marmelos* Correa, A. Chatterjee and J. Bose, *J. Ind. Chem. Soc.* **29**, 425 (1952); *Chem. Abstr* **47**, 10544 (1953); R. N. Chakaravarti and B. Dasgupta, *J. Chem. Soc.* 1580 (1958).

[25] J. F. Fisher, H. E. Nordby, A. C. Waiss Jr. and W. L. Stanley, *Tetrahedron* **23**, 2523 (1967).

[26] J. R. Price, in *Chemical Plant Taxonomy* (Edited by T. Swain) Chapt. 15 Academic Press, London (1963)

[27] J. F. Kefford, *Advances in Food Research IX*, p. 340.

[28] For exceptions, see I. Stewart and T. A. Wheaton, *Science* **145**, 60 (1964); I. Stewart, W. F. Newhall and G. J. Edward, *J. Biol. Chem.* **239**, 930 (1964); T. A. Wheaton and I. Stewart, *Nature, Lond.* **206**, 620 (1965).

[29] R. M. Horowitz in W. B. Sinclair, *The Orange, Its Biochemistry and Physiology* p. 334. University of California Press (1961)

The absence of flavonoids, a class of compounds well known for their complexing ability with borate, may explain the tolerance of *S. buxifolia* to high boron soils,[30] compared to *Citrus* species, which are especially sensitive to boron-containing soils.[31]

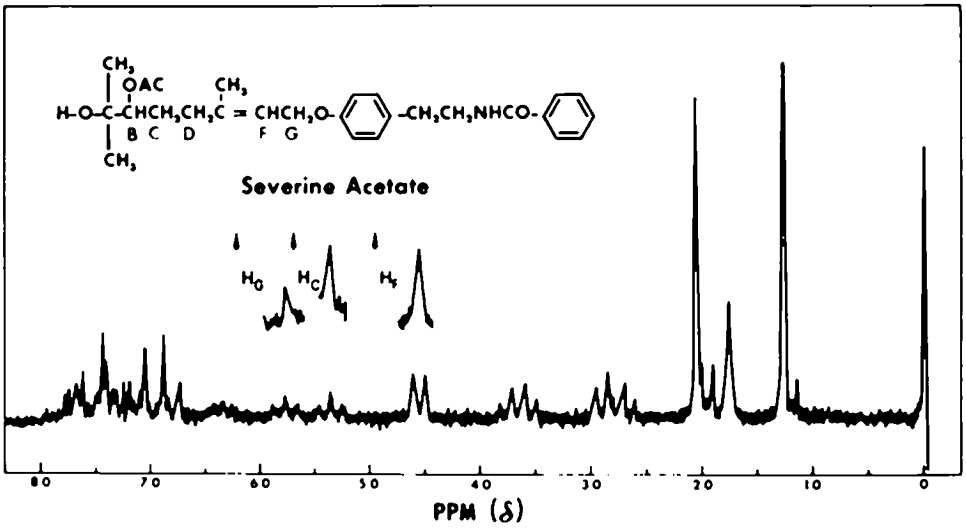


FIG. 1. NMR spectrum of severine acetate (17) in deuteriochloroform.

EXPERIMENTAL[32]

Isolation. The whole branch ends, including the leaves, of *S. buxifolia*, collected at the Citrus Research Center, University of California, Riverside, were dried, ground and extracted with acetone. Solvent was removed from the extracts and the residue chromatographed on alumina. The fractions were monitored by silicic acid TLC using a 1:1 $CHCl_3$:EtOAc solvent system. Waxes and fats were washed from the column with hexane. Seselin was eluted with 30% benzene in hexane m.p. 117 119·5°; λ_{max}^{EtOH} 218 (25,000), 284 (9800), 293 (11,000), 331 (11,500) mμ, NMR δ 7·61 (d) J = 9 H-4, 7·23 (d) J = 8 H-5, 6·83 (d) J = 10 H-1', 6·69 (d) J = 8 H-6, 6·02 (d) J = 9 H-3, 5·72 (d) J = 10 H-2', 1·47 (s) C-methyls ppm. ($CDCl_3$).[33]

Fractions eluted with benzene gave, after removal of solvent, the same alkaloid as that obtained from the fruit, m.p. 113–114° (AcOEt hexane). 1:1 $CHCl_3$ benzene eluted compound 4, m.p. 128 130°, after sublimation and crystallization from AcOEt hexane. ν 1724, 1616 cm^{-1} (Nujol); λ_{max}^{EtOH} 207 (30,500), 252 (7300), 261 (8200), 327 (11,400) mμ. The UV spectrum was unaffected by added base. NMR δ 7·87 (d) J = 10 H-4, 6·30 (s) H-6, 6·60 (d) J = 10 H-3, 3·74, 3·75 (s) OMe, 3·75 (s) α-methylene, 2·78 (p) J = 7 α-methine, 1·10, 1·22 (s) C-Me's ppm ($CDCl_3$); 4·67 (s) α-methylene, 3·36 (s) MeO's 2·55 (p) α-methine, 1·13, 1·02 C-Me's ppm (benzene). (Found: C, 66·4; H, 6·20. $C_{16}H_{18}O_5$ requires: C, 66·19; H, 6·25%.)

2,4-Dinitrophenylhydrazone, m.p. 238–239° (AcOEt hexane). (Found: C, 55·8; H, 4·71. $C_{22}H_{22}N_4O_8$ requires: C, 55·92; H, 4·69%.)

Semicarbazone m.p. 234–238° (dec) from EtOH. (Found: C, 58·7; H, 6·17. $C_{17}H_{21}N_3O_5$ requires: C, 58·76; H, 6·09%.)

Formation of 7. One g of 4 was dissolved in 5% NaOH aq with warming on a steam bath. Alternate

[30] F. M. Eaton and G. Y. Blair, *Plant Physiol.* **10**, 411 (1935); see also Ref. 3, p. 278.

[31] K. Tauböck, *Naturwissenschaften* **30**, 439 (1942).

[32] NMR spectra were taken at 60 Mc/s and are given in δ relative to internal TMS. The relative areas of peaks were consistent with the assignments. The author is indebted to L. M. White for the analytical data.

[33] For IR and NMR curves of seselin see S. N. Shanbhag, C. K. Mesta, M. L. Maheshwari, S. K. Paknikar and S. C. Bhattacharyya, *Tetrahedron* **20**, 2605 (1964).

additions of Et_2SO_4 and 5% NaOH aq were then made over an hr. at about 50° keeping the soln basic. At the end of this period the soln was made strongly basic and boiled for 10 min. The soln was then acidified, cooled and extracted with ether. The ether extracts were washed with 5% Na_2CO_3 aq. The carbonate washings were acidified and the acid fraction collected with ether. Solvent was removed from the acid fraction and the residue dissolved in 5% NaOH aq. To this soln was added 200 ml of 2% $KMnO_4$ aq with frequent shaking over an hr with slight warming on a steam bath. The soln was then filtered, acidified and the filtrates extracted 6x with ether. The ether extracts were concentrated and excess diazomethane in ether added. After 10 min solvent was blown off in an air jet and the residue filtered through a short column of acid-washed alumina with benzene. Solvent was removed from the filtrates and the residue crystallized from ether, m.p. 121 123°; lit.[12] m.p. 125 126°: NMR δ 6·22 (s) aromatic, 3·93 (q) J = 7 ethyl methylene, 3·86 (s) OMe, 3·84 (s) OMe, 1·27 (t) J = 7 ethyl methyl ppm ($CDCl_3$).

Thioketal of **4**. A soln of 1 g of **4**, excess ethylene dithiol, and 2 ml of BF_3 Et_2O in 10 ml of glacial AcOH was allowed to stand 48 hr at room temp. The mixture was decomposed with water and extracted with $CHCl_3$. The extracts were washed with 5% Na_2CO_3 aq and water, dried and concentrated. The concentrated soln was filtered through a short column of alumina. Removal of solvent from the filtrates and crystallization of the residue from AcOEt hexane gave 0·6 g of **8**, m.p. 184 187°; ν 1712, 1611 cm^{-1} (Nujol); λ_{max}^{EtOH} 207, ~254, 260, 326 mμ; NMR δ 7·98 (d) J = 10 H-4, 6·34 (s) H-6, 6·29 (d) J = 10 H-3, 3·96, 3·90 OMe's, 3·44 (s) α-methylene, 3·10 (s) methylene, 1·32, 1·20, 1·25, 1·13 J = 7 C-Me's[34] ppm ($CDCl_3$). (Found: C, 59·3; H, 6·13. $C_{18}H_{22}O_4S_2$ requires: C, 58·98; H, 6·05%.)

5,7-Dimethoxy-8-(3'methylbutyl)coumarin (**9**). A soln of 200 mg of **8** in 95% EtOH was refluxed with 2 g of Raney Ni for 1 hr on a steam bath. The soln was cooled, filtered and solvent removed. The residue was filtered through a short column of alumina with benzene. Solvent was removed from the filtrates and the residue crystallized from AcOEt–hexane to give 45 mg of **9**, m.p. 128 130°; ν 1724, 1612 cm^{-1} (Nujol); λ_{max}^{EtOH} 207, ~255, 261, 326 mμ. (Found: C, 69·5; H, 7·30. $C_{16}H_{20}O_4$ requires: C, 69·54; H, 7·30%.)

From the mother liquors of the desulfuration were isolated 20 mg of a second, more soluble, product m.p. 87 88·5° (hexane) which appeared to be 5,7-dimethoxy-8-(3'-methylbutyl)3,4-dihydrocoumarin. (Found: C, 69·2; H, 7·79. $C_{16}H_{22}O_4$ requires: C, 69·04; H, 7·97%.)

Isolation of the alkaloid **19** *from the fruit*. Extraction of 10 kg of dried and ground fruit with acetone gave 65 g of crude **19** which separated on standing. Further amounts of the alkaloid could be obtained from the mother liquors by chromatography on alumina. The alkaloid was not soluble in cold conc HCl and dissolved only on heating with darkening. Repeated crystallization from benzene gave pure **19** m.p. 113 114°; ν 3330, 1739, 1638, 1577, 1537 cm^{-1} (Nujol); λ_{max}^{EtOH} 226, 272, 282 mμ. (Found: C, 76·2, H, 9·43; N, 2·14. $C_{41}H_{63}NO_5$ requires: C, 75·74; H, 9·77, N, 2·15%.)

Removal of solvent from the mother liquors of the extracts and chromatography of the residue on alumina gave small amounts of imperatorin (**13a**) and seselin (**1**) from the hexane elutants while further amounts of the alkaloid were eluted with 1:1 benzene:hexane. Elution with benzene and benzene chloroform mixtures gave fractions from which bergapten (**13b**) and isopimpinellin (**13c**) were isolated. Identity of **13a**, **13b** and **13c** was established by comparison with authentic samples.

Severine (**16**). A soln of the natural alkaloid in dioxan 5% NaOH aq was refluxed for 1 hr. The cooled soln was extracted with $CHCl_3$. The extracts were dried, concentrated, and filtered through a short column of alumina with $CHCl_3$. Removal of solvent from the filtrates and crystallization of the residue from benzene gave **16**, m.p. 141 143°; ν 3335, 1644, 1540 cm^{-1} (Nujol); λ_{max}^{EtOH} 226 (23,000) 273 (2400), 283 (1600) mμ. Prominent mass spect. peaks occurred at *m/e* 202 (12), 122 (12), 121 (10), 120 (84), 107 (14), 105 (60), 86 (10), 91 (50), 79 (10), 77 (34), 71 (16), 60 (10), 59 (33), 57 (10), 55 (14), 53 (14), 45 (12), 44 (94), 43 (100), 41 (46), 39 (18). (Found: C, 72·9; H, 7·65; N, 3·31. $C_{25}H_{33}NO_4$ requires: C, 72·9; H, 8·08; N, 3·42%.)

Acidification of the base soluble material gave palmitic acid, identified by conversion to its methyl ester with diazomethane and measurement of its retention time by VPC. The palmitic acid was further characterized as its amide and anilide.

Severine acetate (**17**). Acetylation of severine with Ac_2O pyridine on a steam bath for 1 hr gave a monoacetate, m.p. 109 112°; ν 3325, 1750, 1642, 1538 cm^{-1} (Nujol) (Found: C, 71·8; H, 7·40; N, 2·99. $C_{27}H_{35}NO_5$ requires: C, 71·5; H, 7·77; N, 3·08%.)

Lead tetraacetate oxidation of severine. Lead tetraacetate (1 g) was added to a soln of 200 mg **16** in 30 ml of glacial AcOH. The soln was heated in a flask equipped with a distilling head so that volatile material

[34] The isopropyl C-Me resonances appeared as two sets of doublets indicating substantial steric hindrance to free rotation about the 2', 3'-bond.

could be distilled off and collected in 2,4-dinitrophenylhydrazine soln. The yellow ppt which formed was collected and filtered through a short column of alumina with $CHCl_3$. The product, m.p. 124°, was identical with an authentic sample of acetone 2,4-DNP.

Severone (**18**). Jones' reagent was added dropwise to a soln of 0·5 g severine in acetone with ice bath cooling. Addition was halted when the soln showed a permanent chromic acid color. After standing 30 min a large volume of water was added and the mixture extracted with $CHCl_3$. The extracts were dried, solvent was removed, and the residue was filtered through a short column of alumina with benzene. Solvent was removed from the filtrates and the residue crystallized with $CHCl_3$-hexane, m.p. 75–76·5°; ν 3480, 1757, 1740, 1660, 1530 cm^{-1} ($CHCl_3$). (Found: C, 76·6; H, 7·59; N, 3·23. $C_{25}H_{31}NO_4$ requires: C, 76·30; H, 7·94; N, 3·56%.)

Acid hydrolysis of severine. To a soln of 5 g crude severine in 40 ml AcOH was added 10 ml of conc HCl. The mixture was heated on a steam bath for 30 min, during which time the soln turned very dark. The cooled soln was diluted with water and extracted with ether. The ether phase was extracted with 5% NaOH aq and the basic extracts acidified with HCl aq. This aqueous soln was then extracted 2x with ether. The combined ether extracts were dried and solvent removed to give **20**, which was crystallized from AcOEt hexane, m.p. 165–165·5°; ν 3280, 1636, 1542 cm^{-1} (Nujol); λ_{max}^{EtOH} 227 (17,000), 270 (2300), 285 (1635) mμ. NMR δ 7·62 (broad singlet) N-benzoyl, 7·29 (d) J = 9, 6·95 (d) J = 9 aromatic, 3·04 (t) J = 6 benzylic methylene, 3·94 (t) J = 6 amide methylene ppm (CF_3COOH). (Found: C, 74·3; H, 6·27. $C_{15}H_{15}NO_2$ requires: C, 74·7; H, 6·26%.)

The acetyl derivative **21** crystallized from AcOEt–hexane, showed m.p. 121·5–123°; ν 3328, 1757, 1642, 1542 cm^{-1} (Nujol); NMR δ 7·81–7·28 (m) benzoyl, 7·11 (d) J = 9, 6·95 (d) J = 9 aromatic, 2·84 (t) J = 6 benzylic methylene, 3·58 (t) J = 6 amide methylene, 2·22 (s) acetoxy ppm ($CDCl_3$). (Found: C, 71·8; H, 6·09; N, 4·94. $C_{17}H_{17}NO_3$ requires: C, 72·06; H, 6·04; N, 4·94%.)