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PUNCTATIN: A NEW GERMACRADIENOLIDE FROM *LIATRIS PUNCTATA**

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Key Word Index—*Liatris punctata*; Compositae; germacradienolide; sesquiterpene lactone; punctatin; eupatilin.

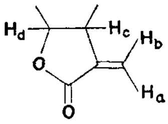
Abstract—The isolation and strutcure determination of punctatin (I), a new germacradienolide from *Liatris punctata* Hook., is reported. The flavone eupatilin was also found.

INTRODUCTION

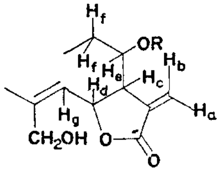
AS PART of a chemical study of the genus *Liatris*, tribe Eupatoriae, family Compositae, we have examined a small collection of *Liatris punctata* Hook., a species widely distributed in the western plains and prairies of North America.[1] The present communication describes isolation and structure determination of a new germacradienolide which we have named punctatin. Eupatilin (5,7-dihydroxy-3′,4′,6′-trimethoxyflavone) was also found.

RESULTS

Punctatin I, $C_{20}H_{24}O_7$ (high resolution MS), m.p. 163–165°; $[\alpha]_{Hg}$ −521°, was a conjugated γ-lactone (IR bands at 1765 and 1655 cm^{-1}, strong UV end absorption). The NMR spectrum exhibited the typical two doublets, H_a and H_b, of partial structure A. Spin decoupling experiments involving H_a and H_b established the location of the narrowly split H_c multiplet at 3·10 ppm. H_d was a doublet of doublets at 5·15 ppm (*vide infra*). The



(A)



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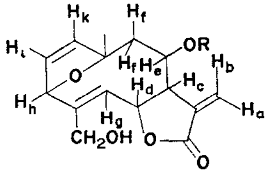

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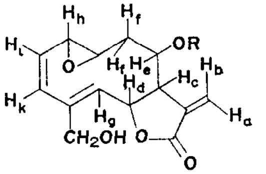

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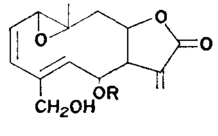

(E)

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* Part II in a series "Constituents of *Liatris* Species". For Part I see HERZ, W. and WAHLBERG, I. (1973) *Phytochemistry*, **12**, 429.

[1] GAISER, L. O. (1946) *Rhodora* **48**, 165, 216, 273, 331, 393. The origin of our collection (Colorado) suggests that it represents *L. punctata* var. *typica*.

presence of partial structure A was confirmed by formation of a dihydro derivative II on $NaBH_4$ reduction. In the NMR spectrum of II the signals of H_a and H_b were replaced by a new methyl doublet.

H_c was also coupled to a complex signal at 5·26 ppm, H_e, which was tentatively assigned to a proton on a carbon carrying an ester group. The presence of the latter was indicated by an IR band at 1722 cm^{-1}. If punctatin were a sesquiterpene lactone, the molecular formula would require a C_5-acid as the ester component. This was corroborated by the MS, which in addition to the molecular peak at m/e 376·1516 displayed diagnostically important peaks at 277·1078 (M-$C_5H_7O_2$), 261·1100 (M-$C_5H_7O_3$), 260·1048 (M-$C_5H_8O_3$), 99·0444 ($C_5H_7O_2$) and 81·0339 (C_5H_5O) compatible with a hydroxyl-containing $C_5H_7O_2$ acyl side chain in I. Acetylation of I afforded a diacetate III, whose MS had corresponding peaks at 460 (M), 400 (M-HOAc), 319 (M-$C_7H_9O_3$), 303 (M-$C_7H_9O_4$), 302 (M-$C_7H_{10}O_4$), 242 (M-$C_7H_{10}O_4$-HOAc), 141 ($C_7H_9O_3$), 99 141-ketene) and 81 (141-HOAc).

The identity of the side chain was revealed by the NMR spectrum of I which contained a vinylic methyl doublet (J 7 Hz), coupled to a slightly broadened quartet at 6·41 ppm. This in turn was long-range coupled to a two proton broadened singlet at 4·17 ppm, apparently due to a $-CH_2OH$ group. This signal, which actually was superimposed on that of another carbinol group, was shifted to 4·62 ppm in the spectrum of III. On the basis of these results and particularly the relative chemical shifts of all these protons it was obvious that punctatin incorporated a sarracinoyl moiety.[2] It is interesting to note that an acetylsarracinoyl side

TABLE 1. NMR SPECTRA OF

Compound	H-1	H-2	H-3	H-5	H-6	H-7
I	3·30*dd* (7·1, 0·5)	5·69*dd* (7·1, 12·0)	6·19*dd* (12·0, 0·5)	5·62*dbr* (10·1, 0·5)	5·15*dd* (10·1, ~1)	3·10*m* ($W_{1/2}=5$)
II	3·33*d* (7)	5·69*dd* (7·12)	6·13*d* (12)	5·84*dbr* (11, 0·5)	5·17*dd* (11, 1)	§
III	3·27*d* (7·3)	5·71*dd* (7·3, 11·5)	6·20*dd* (11·5)	5·66*dbr* (11·3, ~1)	5·09*dd* (11·3, ~1)	3·06*m* ($W_{1/2}$ ~5)
IV	§	§	§	§	4·95*m*	§
V	§	§	§	§	5·02*m* ($W_{1/2}$ ~16)	§

* Run at 90 MHz in $CDCl_3$ solution on a Bruker NMR spectrometer using TMS as internal standard. Values are in ppm. Multiplicities are indicated by the usual symbols: *d*—doublet; *t*—triplet; *q*—quartet; *br*—broadened singlet; *m*—multiplet whose center is given. Unmarked signals are singlets. Figures in parentheses are coupling constants measured by spin decoupling.

† Intensity three protons

‡ Intensity two protons.

§ Obscured signal.

‖ Arbitrary assignment.

¶ Acetates at 2·07 and 2·02 ppm.

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[2] EDWARDS, JR., J. D., MATSUMOTO, T. and HASE, T. (1967) *J. Org. Chem.* **32**, 244.

chain is present in liatrin, sesquiterpene lactone recently isolated from *Liatris chapmanii* (T. + G.) Kuntze,[3] while the corresponding *cis*-isomer has been encountered in other sesquiterpene lactones isolated from different *Liatris* species.[4]

Irradiation of punctatin at the frequency of H_e affected the multiplet due to H_c and collapsed two well-separated doublets of doublets at 2·86 and 1·43 ppm into two doublets (*J* 14·2 Hz). Conversely, irradiation at the frequency of each of these affected the H_e multiplet and collapsed the other into a doublet, demonstrating that H_e was adjacent to a $-CH_2-$ grouping, H_f of partial structure B.

The doublet of doublets at 5·15 ppm in the NMR spectrum of I, assigned to the lactone hydrogen, H_d, was coupled to a broadened doublet at 5·62 ppm, H_g. The broadening was found to be due to long-range coupling to the second carbinol group not part of the sarracinoyl moiety. Hence, H_g is a vinylic proton allylically coupled to a carbinol group (structure B). This assignment was confirmed by the formation of a hydrogenolysis product IV, $C_{29}H_{30}O_5$, on catalytic hydrogenation of I using PtO_2 in HOAc. In the NMR spectrum of IV the signals due to both carbinol groups were replaced by methyl doublets. Furthermore, the IR spectrum of IV had no hydroxyl absorption and the MS displayed prominent peaks at *m/e* 85 (C_5H_9O) and 57 (C_4H_9), most likely derived from the methylbutyroyl side chain.

The NMR spectrum of I also contained a methyl singlet at 1·43 ppm, a doublet at 3·30 ppm (*J* 7·1 Hz) H_h, coupled to a doublet of doublets at 5·69 ppm, H_i, which in turn was coupled to a doublet H_k at 6·19 ppm. These data suggested that H_i and H_k were vinylic

PUNCTATIN DERIVATIVES*

H-8	H-9	H-13	H-14†	H-15‡	H-3‡	H-4	H-5†
5·26*m* ($W_{1/2}$ = 7)	2·86*dd* (14·2, 4·0) 1·43*dd* (14·2, 2·5)	6·40*d* (2·1) 5·77*d* (1·4)	1·43	4·17*br*	4·17*br*	6·41*q* (7·2)	2·04*d* (7·2)
5·26*m* ($W_{1/2}$ = 7)	2·90*dd* (14, 4) §	1·20*d* (7)†	1·38	4·20*br*	4·20*br*	6·40*q* (7)	2·09*d* (7)
5·34*m* ($W_{1/2}$ = 8)	2·84*d* (14·8, 4·0) 1·44*dd* (14·8, 2·9)	6·54*d* (1·8) 5·86*d* (1·7)	1·41	4·62*br*	4·62*br*	6·53*q* (7)	2·08*d*¶ (7)
5·20*m*	§	1·15*d*†‖ (6·5)	1·46	1·12*d*‡,‖ (7·5)	1·14*d*†,‖ (6·5)	§	0·92*t* (7)
5·26*m* ($W_{1/2}$ = 8)	2·71*dd* (15, 4) §	1·14*d* (7)†	1·47	3·58*br*‖	3·76*br*‖	§	0·97*t* (7)

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[3] Kupchan, S. M., Davis, V. H., Fujita, T., Cox, M. R. and Bryan, R. F. (1971) *J. Am. Chem. Soc.* **93** 4916.

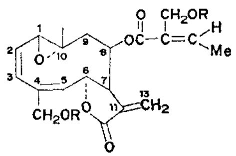
[4] Herz, W. and Wahlberg, I. unpublished results.

protons attached to a disubstituted double bond. In harmony with this, catalytic hydrogenation of I using Pd–C in EtOAc resulted in the uptake of 4 mol eq. of H_2 and afforded a saturated diol V, $C_{20}H_{32}O_7$. Simultaneously the NMR signals due to H_a, H_b, H_g, H_i and H_k disappeared.

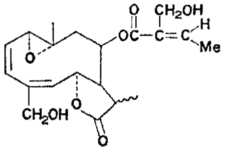
In view of the molecular formula, the NMR data and the absence of absorption due to a ketone group in the IR spectrum of I, the remaining oxygen atom was most likely incorporated in an ether function. Although the multiplicity of the NMR signals of H_h, H_i and H_k would have allowed formulation of punctatin as C with a 3,10 ether bridge, the magnitude of the observed coupling constants between H_i and H_k (J 12 Hz) clearly excluded this alternative (expected $J_{i,k} \sim 7$ Hz[3]), thus leaving structure D which incorporates a 1,10 epoxide function for punctatin.

The presence of the conjugated diene system required by this formula was supported by the UV spectrum which had a shoulder at 232 nm ($\epsilon \sim 18000$), superimposed on very strong end absorption due to combination of the conjugated lactone (maximum expected at 205–210 nm) with the conjugated ester chromophore (maximum expected near 215 nm). Similarly, while the UV spectrum of II exhibited maximum absorption at 216 nm (conjugated ester), the band was quite asymmetrical and skewed to longer wave length ($\epsilon_{235} \sim 9000$) due to the presence of the diene. The hypsochromic shift of the diene absorption can be attributed to deviation of the diene chromophore from planarity (*vide infra*).

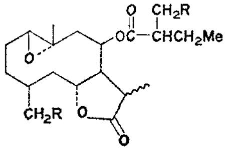
The results so far have been discussed in terms of a formula involving lactone closure towards C-6 and not toward C-8 (structure E). These alternatives could not be distinguished by chemical means since attempted partial hydrolysis using aq. K_2CO_3 in methanol resulted in formation of an 11,13-methanol adduct and treatment with KOH or acid caused extensive decomposition. However, the relative chemical shifts of H_d at 5·02 and H_e at 5·26 ppm in the perhydroderivative V favored lactone ring closure toward C-6, since in comparable substances of established structure, the signal of hydrogen on the carbon carrying the ester group is invariably downfield from the signal of the lactone hydrogen.



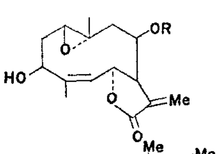
(I) R = H
(III) R = Ac



(II)



(IV) R = H
(V) R = OH



(VIa) R = $-CO-C(Me)=C(Me)H$
(VIb) R = $-CO-CH(Me)-Me$

A comparison of the NMR spectra of punctatin, heliangin (VIa)[5] and erioflorin (VIb)[8] proved instructive. In fact the coupling constants of H-5, H-6, H-7 and H-8 were very similar, indicating not only that the lactone ring of punctatin was also closed to C-6 but

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[5] Nishikawa, N , Kamiya, K., Takabatake, A , Oshio, H., Tomiie, Y and Nitta, I (1966) *Tetrahedron* **22**, 3601 Contrary to its depiction as a *trans*-Δ^4-germacrenolide, this substance has a *cis*-C-4, C-5 double bond.[6,7]

[6] Herz, W. and Bhat, S. V (1972) *J Org. Chem.* **37**, 906, footnote 15.

[7] Neidle, S. and Rogers, D. (1972) *Chem. Commun* 140.

[8] Torrance, S J , Geissman, T. A. and Chedekel, M. R. (1969) *Phytochemistry* **8**, 2381 The correlation with heliangine carried out by these authors requires that erioflorin be reformulated as a *cis*-C-4, C-5 germacrenolide.[6,7]

also that the stereochemistry of heliangine, erioflorin and punctatin was identical at these positions.

The similarity was confirmed in the following manner. (a) Irradiation at the frequency of the two $-C\underline{H}_2O$ signals in the NMR spectrum of punctatin produced a 14% increase in the strength of the H-5 signal and an 11% increase in the strength of the H-3 signal. Hence the C-4, C-5 and the C-2, C-3 double bonds are *cis*. Simultaneously a 12% enhancement in the integrated absorption of the side chain vinyl quartet (H-3′) confirmed the orientation around the double bond of the sarracinoyl residue. (b) Irradiation at the frequency of the methyl singlet produced a 15% increase in the strength of the H-9a signal, but no enhancement in the strength of the signal of H-1. Hence the epoxide ring of punctatin possesses the *trans*-configuration. This conclusion was corroborated by the existence of a strong NOE between the methyl group and H-6 (13%), an observation which requires that the methyl group be oriented toward H-6 and the oxide function away from it. In a *cis,cis*-$\Delta^{2,4}$-germacradiene this is only possible (model) if the epoxide is *trans*; the model also indicates that the diene system is highly skewed, thus accounting for the blue shift of the UV absorption.[9]

The diene chromophore also seems to exert a dominant effect on the shape on the CD curve which exhibited only one very strong minimum at 225 nm ($[\theta] \sim -140000$), the much weaker Cotton effect due to the n–π^* transition of the α,β-unsaturated lactone function usually found near 25 nm[10] seemingly having been swamped. The molecular ellipticity of II is somewhat reduced (λ_{max} 225 nm, $[\theta]$ -60000), but still unusually strong.

EXPERIMENTAL

M.ps are uncorrected. Rotations were run in $CHCl_3$, UV spectra in MeOH, IR spectra as KBr pellets, and CD curves in MeOH. High-resolution MS were run at 70 eV on a MS-9 mass spectrometer. Analyses were performed by F. Pascher, Bonn, Germany.

Isolation of punctatin and eupatilin. Ground above-ground *Liatris punctata* Hook., wt 0·7 kg, collected by Dr. B. H. Braun in August 1960 in the vicinity of Boulder, Colorado, was extracted with $ChCl_3$ and worked up in the usual manner.[15] The crude gum, wt 16 g, was chromatographed over 225 g of silicid acid (Mallinckrodt, 100 mesh), 200 ml fractions being collected in the following order: 1–15 (C_6H_6), 16–25 (C_6H_6–$CHCl_3$, 4:1), 26–35 (C_6H_6–$CHCl_3$, 1:1), 36–45 (C_6H_6–$CHCl_3$, 1:4), 46–75 ($CHCl_3$), 76–85 ($CHCl_3$–MeOH, 99:1), 86–95 ($CHCl_3$–MeOH, 49:1), 96–110 ($CHCl_3$–MeOH, 97:3), 111–115 ($CHCl_3$–MeOH, 19:1), 116–125 ($CHCl_3$–MeOH, 9:1). Fractions 28–30 were combined and recrystallized from MeOH to give eupatilin (5,7-dihydroxy-3′,4′,6′-trimethoxyflavone), wt 54 mg, which melted at 233–235° (lit.[16] 234–236°),

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[9] For a similar reason the UV maximum of a dienone derived from erioflorin is found at considerably shorter wave length than calculated.[8]

[10] Stöcklin, W., Waddell, T. G. and Geissman, T. A. (1970) *Tetrahedron* **26**, 2397. In analogy with the Cotton effects exhibited by heliangenol, woodhousin[6] and orizabin,[6] the sign of the lactone n, π^* Cotton effect in punctatin might be expected to be positive, unless the chirality of the lactone system is affected by the extra double bond at C-2, C-3. Conversely, the possibility of interaction between the lactone function and the diene moiety, coupled with the presence of a second allylic oxygen on the other terminus of the system renders questionable[11–13] the applicability of the homoannular cisoid diene chirality rule[14] to the present situation.

[11] Beecham, A. F., McL. Mathieson, A., Johns, S. R., Lamberton, J. A., Sioumis, A. A., Batterham, T. J. and Young, I. G. (1971) *Tetrahedron* **27**, 3725.

[12] Beecham, A. F. (1972) *Tetrahedron* **27**, 5207.

[13] Charney, E., Edwards, J. M., Weiss, U. and Ziffer, H. (1972) *Tetrahedron* **28**, 973.

[14] (a) Moscowitz, H., Charney, E., Weiss, U. and Ziffer, H. (1961) *J. Am. Chem. Soc.* **83**, 4661; (b) Weiss, U., Ziffer, H. and Charney, E. (1965) *Tetrahedron* **21**, 3105; (c) Charney, E. (1965) *Tetrahedron* **21**, 3127.

[15] Herz, W. and Högenauer, G. (1962) *J. Org. Chem.* **27**, 905.

[16] Kupchan, S. M., Sigel, C. W., Hemingway, R. J., Knox, J. R. and Udayamurthy, M. S. (1969 *Tetrahedron* **25**, 1603.

UV and NMR spectra identical with the reported spectra, IR spectrum superimposable on that of an authentic sample; m.m.p. was undepressed. Fractions 54–70, which showed a major spot on TLC, were combined and recrystallized from isopropylether–MeOH to yield punctatin, 1·5 g, m.p. 163–165°; $[\alpha]_{Hg}$ $-521°$ (c 0·126), λ_{max} 232 nm ($\epsilon \sim 17600$); strong end absorption at 210 nm; CD curve λ_{max} 225 nm ($[\theta]$ -140000), $[\theta]_{240}$ -46500, $[\theta]_{245}$ -31000, IR bands at 3550, 3450, 1765, 1720 and 1655 cm^{-1}; *m/e* (composition, %): 376 (M, $C_{20}H_{24}O_7$, 0·4), 277 ($C_{15}H_{17}O_5$, 2), 260 ($C_{15}H_{16}O_4$, 4), 242 ($C_{15}H_{14}O_3$, 3), 99 ($C_5H_7O_2$, 100) and 81 (C_5H_5O, 17). (Calc for $C_{20}H_{24}O_7$: C, 63·82; H, 6·43; O, 29·75. Found: C, 63·55, H, 6·58; O, 29·81.)

Dihydropunctatin (II). To a solution of 54 mg of I in 5 ml MeOH was added with stirring 34 mg of $NaBH_4$ in 1 ml MeOH at 0°. Stirring was continued for 1·5 hr at 0°. The solution was acidified, evaporated at reduced pressure, diluted with H_2O and extracted with $CHCl_3$ Chromatography over silica gel (hexane–EtOAc, 1·3) gave 21 mg of a gum (II), λ_{max} 216 nm (ϵ 12800) (232 nm $\epsilon \sim 9000$); IR bands (neat) at 3400, 1760, 1720 and 1650 cm^{-1}, CD curve λ_{max} 225 nm ($[\theta]$ -60000), $[\theta]_{240}$ -40000, $[\theta]_{245}$ -29000, *m/e* (composition, %): 378 ($C_{20}H_{26}O_7$, 2), 279 ($C_{15}H_{19}O_5$, 20), 262 ($C_{15}H_{18}O_4$, 28), 224 ($C_{15}H_{16}O_3$, 14), 99 ($C_5H_7O_2$, 100) and 81 (C_5H_5O, 57) (Calc. for $C_{20}H_{26}O_7$: MW 378·1678. Found: MW–MS, 378·1673.)

Punctatin diacetate (III) 65 mg of I was acetylated with Ac_2O–pyridine. Chromatography over silica gel (hexane–EtOAc, 4:1) afforded 55 mg of III, which after recrystallization from isopropylether–MeOH had m.p. 105–107°, IR bands at 1755, 1728 and 1660 cm^{-1}, *m/e* (composition, %): 460 ($C_{24}H_{28}O_9$, 1), 400 ($C_{20}H_{24}O_7$, 1), 319 ($C_{17}H_{19}O_6$, 3), 302 ($C_{17}H_{18}O_5$, 11), 260 ($C_{15}H_{16}O_4$, 7), 242 ($C_{15}H_{14}O_3$, 22), 141 ($C_7H_9O_3$, 80), 99 ($C_5H_7O_2$, 54) and 81 (C_5H_5O, 100). (Calc. for $C_{24}H_{28}O_9$: C, 62·60, H, 6·13, O, 31·27. Found: C, 62·71; H, 6·25; O, 30·79%)

Preparation of IV. A solution of 76 mg of I in 5 ml of HOAc was reduced at atmospheric pressure with 20 mg of platinum oxide for 5 hr. The filtered solution was evaporated and chromatographed over silica gel (hexane–EtOAc, 3:1) The product, IV, was recrystallized from hexane–EtOAc: yield 8 mg, m p. 163–165°; IR bands at 1765 and 1735 cm^{-1}; *m/e* (composition, %): 352 ($C_{20}H_{32}O_5$, 0·1), 324 ($C_{19}H_{32}O_4$ and $C_{18}H_{28}O_5$, 0·2), 267 ($C_{15}H_{23}O_4$, 2), 250 ($C_{15}H_{22}O_3$, 7), 85 (C_5H_9O, 100) and 57 (C_4H_9, 20) (Calc. for $C_{20}H_{32}O_5$: MW, 352·2250. Found: MW–MS, 352·2253)

Octahydropunctatin (V). A solution of 50 mg of I in 8 ml EtOAc was stirred with 25 mg of 5% Pd–C in H_2 for 4 hr, filtered and evaporated. The residue was chromatographed over silica gel (hexane–EtOAc, 1:1) to give 38 mg of V. Recrystallization from isopropylether–EtOAc afforded material, which melted at 136–141°, IR bands at 3430, 1765 and 1725 cm^{-1} (Calc. for $C_{20}H_{32}O_7$: C, 62·48, H, 8·39, O, 29·13 Found: C, 62·13; H, 8·45; O, 29·18%)

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