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PERINAPHTHENONE PIGMENTS FROM THE FRUIT CAPSULES OF *LACHNANTHES TINCTORIA*[1]

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Abstract—The seed capsules of *Lachnanthes tinctoria* (Haemodoraceae) contain lachnanthocarpone (IV), a non-glycosidic 9-phenylperinaphthenone; two other related pigments have also been isolated. The only other perinaphthenone found in a higher plant is the glycoside haemocorin (I) from Australian *Haemodorum* sp.

INTRODUCTION

Lachnanthes tinctoria (Walt.) Ell. [*L. caroliniana* (Lam.) Dandy], "red-root", a plant locally common in acid bogs, particularly of the Pine Barrens of New Jersey, is the only generally recognized[2] representative of the small monocotyledonous family, Haemodoraceae, to be found anywhere in the northern hemisphere above lat. 30° N., the other genera occurring in Australia, South Africa, and South and Central America.

Many species of this family are characterized by colorful root systems; the bright-red tubers of Australian *Haemodorum* sp. have been found by Cooke *et al.*[3] to contain the glycoside haemocorin (I), the only perinaphthenone pigment found so far in a higher plant. Investigation of *L. tinctoria* was prompted by its status as a geographically isolated representative of a family known to produce a chemically unique pigment; furthermore, the plant has long been suspected[4] to contain photodynamically active constituents. This suspicion goes back to the report of an American correspondent, quoted in Darwin's *Origin of Species*,[5] that farmers in areas where *L. tinctoria* is common, raise only black pigs, which seem more resistant to poisoning by the plant (pink coloration of bones, dropping of hoofs, etc.) than white ones.

Preliminary results have confirmed the occurrence of photodynamically active constituents,[6] and chemical examination has yielded a number of new pigments, most of them perinaphthenones related to I. A note[1] on the isolation of several of these and on a novel photochemical transformation of two of them, has appeared, and a report on the root pigments is in preparation.[7] Results of an independent chemical examination of the aerial parts of the plant have been presented by Cooke *et al.*[8] We wish to describe here the isolation of several new colored substances from the deep brownish red pulp surrounding the seeds.

[1] U. WEISS and J. M. EDWARDS, Paper II on "Pigments of *L. tinctoria* Ell."; Paper I: *Tetrahedron Letters* 4325 (1969).

[2] *Lophiola americana* (Pursh) Wood (*L. aurea* Ker.), "Golden Crest", a plant growing in the New Jersey Pine Barrens often in close association with *Lachnanthes*, and *L. septentrionalis* Fern. of Nova Scotia, are placed in the Haemodoraceae in some classifications, while others include them in the tribe Conostylidae of the Amaryllidaceae (see J. M. EDWARDS, J. A. CHURCHILL and U. WEISS, *Phytochem.* **9**, 1563 (1970).

[3] R. G. COOKE and W. SEGAL, *Australian J. Chem.* (a) **8**, 107 (1955); (b) **8**, 413 (1955), (c) **11**, 230 (1958).

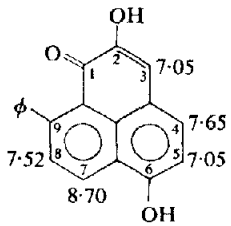
[4] H. F. BLUM, *Photodynamic Action and Diseases Caused by Light*, p. 161, Hafner, New York (1964).

[5] C. DARWIN, *The Origin of Species*, p. 11, Encyclopaedia Britannica Inc., Chicago (1952).

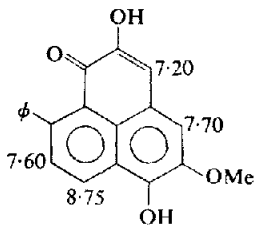
[6] D. P. TSCHUDY and H. L. BLONKOWSKY, unpublished observations.

[7] J. M. EDWARDS and U. WEISS, manuscript in preparation.

[8] R. G. COOKE, to be published in *Phytochem.*, personal communication.



(IVa)



(VIIa)

RESULTS

Thin-layer chromatography (SiO_2, ethyl acetate) of an ethyl acetate extract of the seed capsules showed the presence of four major colored bands: [A] R_f, 0·95; a non-polar yellow fraction which was shown to be a mixture of α-carotene (II) and tristearin (III); [B] R_f, 0·75; lachnanthocarpone (IV); [C] R_f, 0·5; a yellow perinaphthenone (V) isomeric with IV; and [D] R_f, 0·04; a polar yellow substance (VI). Bands A, C, and D are also present in similar extracts of *Lachnanthes* flowers, but here pigment IV is missing. On TLC plates the more polar compounds show characteristic color reactions with NH_3 vapor: B, dark blue; C and D, red.

Compound IV, a brown-red crystalline solid $C_{19}H_{12}O_3$, m.p. 210–215°, gives a deep-green color with $FeCl_3$ and a bright-red solution in conc. H_2SO_4. It is soluble in NaOH, giving a blue solution which is unchanged on addition of Na_2SO_4; this behavior is typical of phenolic perinaphthenones and is very similar to the analogous reactions of the aglycone (VII) of I. With $CuSO_4$ IV forms a stable, black, insoluble complex (from which it can be regenerated by acidification), indicating the presence of two oxygen functions in an *ortho*- or *peri*-relationship. The i.r. spectrum of IV shows major bands at 3630 (non-bonded OH), 3450 (broad), 1625, and 1610 cm^{-1}. The last three of these are very similar to the corresponding bands in VII (3380, 1625 sh and 1610 cm^{-1}) and strongly suggest the presence of an enolic α-diketone grouping, similar to the one of VII. The u.v. spectrum of IV (see Experimental) is likewise very similar to that of VII (λ_{max} 505, 372·5, 355, 300, 277·5 and 250 nm; log ε, 3·71, 3·72, 3·72, 4·23, 4·36 and 4·20), and the differences are consistent with the lack of a methoxyl in IV; an even closer resemblance is found between the spectra of the diacetate of IV and synthetic 6-acetoxy-7(or 9)-phenylperinaphthenone (VIII). [VIIIa and b are tautomers.]

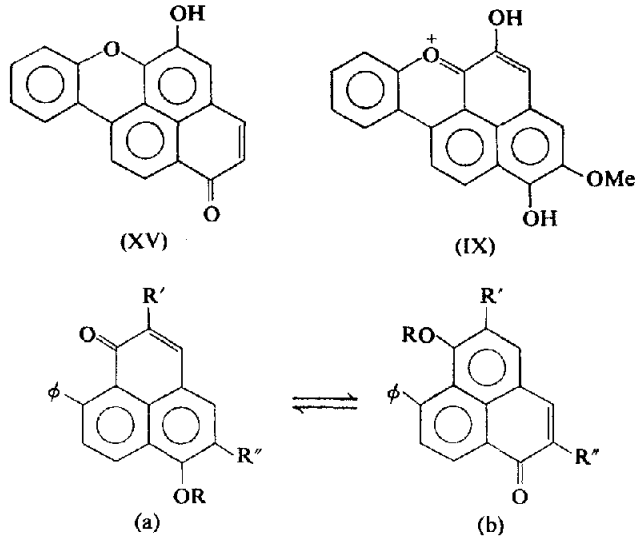
The spectroscopic evidence thus strongly suggests that IV and I, occurring in closely related plants, have the same skeleton and differ only in functionality; this interpretation is supported by all further evidence. Chemical degradation of IV and its derivatives, by processes which were used successfully in the case of VII,[5] proved surprisingly unrewarding.

The *peri*-relationship of the carbonyl to the unsubstituted phenyl group is demonstrated by the mass-spectrum of IV, which has its base peak at $(M\text{-}1)^+$. In the analogous case of VII, this peak has been attributed to the species IX.[9] The same phenomenon has been observed in the spectrum of one of the dimethyl ethers (Xa) of VII,[9] in the spectra of 6-methoxy-9-phenylperinaphthenone (XI) and of one of the dimethyl ethers (XII) of IV (see below). The NMR spectrum of IV shows the unsubstituted phenyl ring (singlet, δ 7·38) and the presence of five other aromatic protons: two AB quartets and a singlet coupled to the downfield pair of one of the quartets by $J = 1$ Hz. The assignments in δ values are shown in the figure

[9] E. S. WAIGHT, in *Some Newer Physical Methods in Structural Chemistry* (edited by R. BONNETT and J. G. DAVIS), p. 67, United Trade Press, London (1967).

together with the chemical shifts of the corresponding protons in VII. The strong deshielding effect of the 6-hydroxyl group on the C-7 proton seems quite characteristic for compounds of this type; a similar effect has been noted, e.g., in some hydroxychromenes.[10] There are no resonances typical of the C-2 and C-3 protons found in perinaphthenone (δ 6·60, and 7·90; $J = 10$ Hz) or in XI (δ 6·88, and 7·70; $J = 9{\cdot}5$ Hz). Although IV is demonstrably capable of tautomerism (formation of *two* isomeric dimethyl ethers), its NMR spectrum remains essentially unchanged between −40 and 100°; the hydrogen bonding between the carbonyl and C-2 hydroxyl group, and the possibility of $p \rightarrow \pi$ overlap between the carbonyl oxygen and the phenyl ring presumably both contribute to the stability of isomer IVa.

Brief treatment of IV with diazomethane gives the two isomeric dimethyl ethers, one red (XII), showing an $(M\text{-}1)^+$ base peak in its mass spectrum, the other orange (XIII), lacking an



(I) (a form only); R′ = *O*-Cellobiose, R″ = OMe, R = H
(IV) (a and b form); R′ = OH, R″ = H, R = H.
(VII) (a and b form); R′ = OH, R″ = OMe, R = H.
(VIII) (a or b form); R′ = H, R″ = H, R = Ac.
(Xa and Xb) R′ = OMe, R″ = OMe, R = Me.
(XI) (a form only); R′ = H, R″ = H, R = Me.
(XII) (a form only); R′ = OMe, R″ = H, R = Me.
(XIII) (b form only); R′ = OMe, R″ = H, R = Me.
(XIV) (b form only); R′ = H, R″ = H, R = Me.

$(M\text{-}1)^+$ peak. The ratio of XII to XIII is about 10:1. The red dimethyl ether is presumed to have structure XII because the $(M\text{-}1)^+$ peak in the mass spectrum implies a *peri*-relationship between the carbonyl group and the phenyl ring; the orange dimethyl ether therefore must have structure XIII. These assignments are supported by the observed solvent shifts in the NMR signals of the —OMe resonances: both methoxyl signals of the red ether shift up-field by $\Delta\delta = -0{\cdot}7$ ppm when C_6H_6 is added to the NMR sample in $CDCl_3$; similar treatment of the sample of XIII caused only one methoxyl signal (that at lower field) to move up-field. Since it is known[11] that such shifts of an aromatic methoxyl signal require that at least one position *ortho* to the group be unsubstituted, the observed shifts are quite consistent with the proposed structures. The up-field position of one of the —OMe resonances in the spectrum of XIII (δ, 3·2) is due to the shielding effect of the phenyl ring. The effect of this *peri*-shielding

[10] A. Arnone, C. Cardillo, L. Merlini and R. Mondelli, *Tetrahedron Letters* 4201 (1967).
[11] H. M. Fales and K. S. Warren, *J. Org. Chem.* **32**, 501 (1967).

is also shown in the spectra of one of the dimethyl ethers (Xb) of VII,[12] and of 6-methoxy-7-phenylperinaphthenone (XIV); a similar up-field resonance has been observed for the 5-methoxyl group in some 4-phenylcoumarins.[13] Finally, the spectrum of the orange ether contains resonances analogous to the C-2 and C-3 protons of perinaphthenone (see above).

That IV is the correct structure for lachnanthocarpone is further supported by the formation of a strongly fluorescent photoproduct,[1] 5-hydroxynaphtho[8,1,2-jkl]xanthenone (XV); the formation of XV parallels the generation of the ionic species (IX) in the mass spectrometer.

The yellow, crystalline compound (V) is an isomer of lachnanthocarpone and its qualitative reactions and spectra (see Experimental) indicate that it, too, is a 2-hydroxyperinaphthenone; the compound forms a single dimethyl ether and does not have an $(M-1)^+$ base peak in its mass spectrum. The available data do not permit a decision to be reached between several possible structures and since chemical degradation was unprofitable in the case of IV, the X-ray analysis of V is being undertaken.

The determination of the structure of VIII has not yet been possible due to lack of material, but preliminary results indicate that it may not be a phenolic perinaphthenone, since it gives a red color with aq. alkali instead of the expected blue; the red-orange color generated by treatment with NH_3 vapor would be compatible with an aurone or chalcone structure.[14]

EXPERIMENTAL

Isolation of the Pigments

The seed capsules, collected near Atzion, New Jersey, in August, were steeped in EtOAc for a week. The solvent was filtered off and the seeds were extracted once more with EtOAc in a Waring Blendor. The combined extracts were taken to dryness and the resulting brown solid was extracted with benzene, leaving a large amount of dark, insoluble material (not yet investigated). From the benzene extract, IV was obtained directly by precipitation with hexane and further purification as described below. Alternatively, chromatography over silica gel, with benzene and increasing proportions of ethyl acetate as eluting solvent, gave compounds II–VI.

Isolation of II and III

Pure benzene eluted the least polar, yellow fraction which, on evaporation, gave a mixture separable by crystallization from methanol. The resulting colorless solid was identified as tristearin (m.p. 52°, lit.[15] ~55°) by comparison with an authentic sample (i.r., NMR and mixed m.p.) and v.p.c. of the methyl ester, obtained after saponification, together with an authentic sample of methyl stearate.

The yellow supernatant was evaporated, freed of fat by saponification and identified as α-carotene by its u.v. spectrum in hexane (λ_{max} 419, 445 and 475 nm; lit.[16] 420, 445, and 475 nm) and co-chromatography with an authentic sample (alumina-impregnated paper, hexane).[17]

Isolation of IV

Compound IV, whether obtained from the column or directly from hexane precipitation, was recrystallized from benzene–hexane and sublimed under vacuum (200°, 0·1 mm) to give *lachnanthocarpone* as a brown solid, m.p. 210–215°; ν_{max}: 3630, 3450, 1625 and 1610 cm^{-1}; λ_{max}: 465, 361, 348, 300, 264 and 242 nm (log ε 3·71, 3·72, 3·72, 4·23, 4·36 and 4·20); δ 8·70 (*d*, $J = 8·5$ Hz; 1H), 7·65 (*d*, $J = 8$ Hz; 1 H), 7·52 (*d*, $J = 8·5$ Hz; 1H), 7·48 (*s*; 5H), 7·05 (*d*, $J = 8$ Hz; 1H), 7·05 (*s*; 1H). (Found: C, 78·95; H, 4·43%; M, 288. $C_{19}H_{12}O_3$ required: C, 79·16; H, 4·20%; M, 288.)

[12] R. THOMAS, personal communication.
[13] S. K. MUKERJEE, T. SAROJA and T. R. SESHADRI, *Tetrahedron* **24**, 6527 (1968).
[14] M. K. SEIKEL, in *Biochemistry of Phenolic Compounds* (edited by J. B. HARBORNE), p. 33, Academic Press, London (1964).
[15] *Dictionary of Organic Compounds* (edited by J. R. A. POLLOCK and R. STEVENS), p. 3203, Oxford University Press, New York (1965).
[16] T. W. GOODWIN, in *Modern Methods of Plant Analysis* (edited by K. PAECH and M. V. TRACEY), Vol. 3, p. 272, Springer Verlag, Berlin (1955).
[17] A. JENSEN and S. L. JENSEN, *Acta Chem. Scand.* **13**, 1863 (1959).

Acetylation of IV with Ac_2O in pyridine gave a yellow *diacetate*, recrystallized from cyclohexane and sublimed (180°, 0·1 mm), m.p. 245–247°; ν_{max}: 1775 and 1655 cm^{-1}; λ_{max}: 400, 363, 346, 324·5 and 257 nm (log ϵ 3·53, 3·60, 3·51, 3·42, and 4·02); δ 8·27 (*d*, $J = 8·5$ Hz; 1H), 7·76 (*d*, $J = 7·5$ Hz; 1H), 7·58 (*d*, $J = 8·5$ Hz; 1H), 7·43 (*s*, 1H), 7·38 (*s*; 5H), ~7·37 (*d*, $J \sim 7$ Hz), 2·52 (*s*; 3H), 2·26 (*s*; 3H). (Found: C, 73·88; H, 4·62%; M, 372. $C_{23}H_{16}O_5$ required: C, 74·18; H, 4·33%; M, 372.)

Methylation of (IV) was achieved either by the action of an excess of CH_2N_2, or by reaction with Me_2SO_4 in acetone in the presence of base. Normal work-up gave in either case a mixture of dimethyl ethers which could be separated by chromatography in benzene over neutral alumina. The more polar *red dimethyl ether* (XII), recrystallized from benzene and sublimed (150°, 0·1 mm), was a bright-orange solid, m.p. 217–218°; ν_{max}: 1642 and 1590 cm^{-1}; λ_{max}: 457, 366, 348, 327 sh., 302, 291, 262 and 243 nm (log ϵ 3·79, 3·53, 3·52, 3·67, 4·03, 4·04, 4·20 and 4·23); δ 8·61 (*d*, $J = 8·5$ Hz; 1H), 7·51 (*d*, $J = 8$ Hz; 1H), 7·50 (*d*, $J = 8·5$ Hz; 1H), 7·37 (*s*; 5H), 6·83 (*d*, $J = 8$ Hz; 1H), 6·78 (*s*; 1H), 4·00 (*s*; 3H), 3·82 (*s*; 3H). (Found: C, 79·64; H, 5·17%; M, 316. $C_{21}H_{16}O_3$ required: C, 79·73; H, 5·10%; M, 316.) The less polar *orange dimethyl ether* (*XIII*) was recrystallized from cyclohexane. The solid had m.p. 198–203° and showed pink fluorescence under u.v. light; ν_{max}: 1648 cm^{-1}; λ_{max}: 448, 368, 350 and 266 nm (qualitative spectrum only); δ 8·60 (*d*, $J = 8·5$ Hz; 1H), 7·80 (*d*, $J = 10$ Hz; 1H), 7·55 (*s*; 1H), 7·55 (*d*, $J = 8·5$ Hz; 1H), 7·4 (*s*; 5H), 6·70 (*d*, $J = 10$ Hz; 1H), 4·00 (*s*; 3H), 3·20 (*s*; 3H). (Found: M = 316·10972. $C_{21}H_{16}O_3$ required: M = 316·10994.)

Isolation of V and VI

Compound V was obtained from the silica column with EtOAc–benzene (3:1) as a yellow solid. The compound was always contaminated with IV and was hard to purify. A far better product was obtained by a similar chromatography of an extract of *Lachnanthes* flowers which contains no IV. On evaporation of the solvent and trituration of the solid residue with methanol, V was obtained as a yellow solid, sublimed (270°, 0·1 mm), m.p. 339–345 (decomp.). ν_{max} 3220, 1618 and 1610 cm^{-1}. λ_{max} 4·38, 321, 266 and 235 nm (log ϵ 3·95, 3·97, 4·03 and 4·24). (Found: M = 288·0784. $C_{19}H_{12}O_3$ required: M = 288·0786.) The solution of V in NaOH is a dark violet color, unchanged on addition of dithionite. Conc. H_2SO_4 gives a red solution.

The final compound (VI) is eluted from the silica column with EtOAc–methanol (1:1). The yellow solid can be recrystallized from ethanol; it is red in NaOH solution and in conc. H_2SO_4.

6-*Methoxy*-9-*Phenylperinaphthenone* (*XI*)

6-Hydroxy-7(or 9)-phenylperinaphthenone was prepared as described by Cooke *et al.*[3c] and sublimed (140°, 0·1 mm); red solid, m.p. 287–290° (lit.[3c] 282–284°). Methylation with CH_2N_2 gave a mixture of monomethyl ethers which could be separated by consecutive chromatography over silica and alumina in benzene. The major product (XI), recrystallized from hexane, is a yellow solid, m.p. 174–177°; δ 8·60 (*d*, $J = 8·5$ Hz; 1H), 7·70 (*d*, $J = 9·5$ Hz; 1H), 7·58 (*d*, $J = 8$ Hz, 1H), 7·54 (*d*, $J = 8·5$ Hz; 1H), 7·39 (*s*; 5H), 6·88 (*d*, $J = 9·5$ Hz; 6·50 (*d*, $J = 8$ Hz; 1H), 4·10 (*s*; 3H). (Found: M = 286·09928. $C_{20}H_{14}O_2$ required: M = 286·09937.) The minor product could not be isolated, but its presence and constitution (XIV) were clear from the NMR spectrum of the mixture of the methyl ethers. The resonances attributable to XIV were: δ 8·68 (*d*, $J = 8·5$ Hz; H-9), 7·7 (*d*, $J = 10$ Hz; H-3) 7·55 (*d*), 7·53 (*d*), 7·43 (*s*; phenyl ring), 6·83 (*d*), 6·66 (*d*, $J = 10$ Hz; H-2), 3·57 (*s*; OMe). Acetylation of the phenol with Ac_2O in pyridine gave 6-acetoxy-7(or 9)phenylperinaphthenone (VIII) as a red-brown solid, m.p. 158–162°, from hexane. λ_{max}: 393, 366, 346, 325 and 257 nm (log ϵ 3·62, 3·55, 3·45, 3·40 and 4·08). (Found: M = 314·09434. $C_{21}H_{14}O_3$ required: M = 314·09429.)

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