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PHENALENONE PIGMENTS OF THE FLOWERS OF *LACHNANTHES TINCTORIA**

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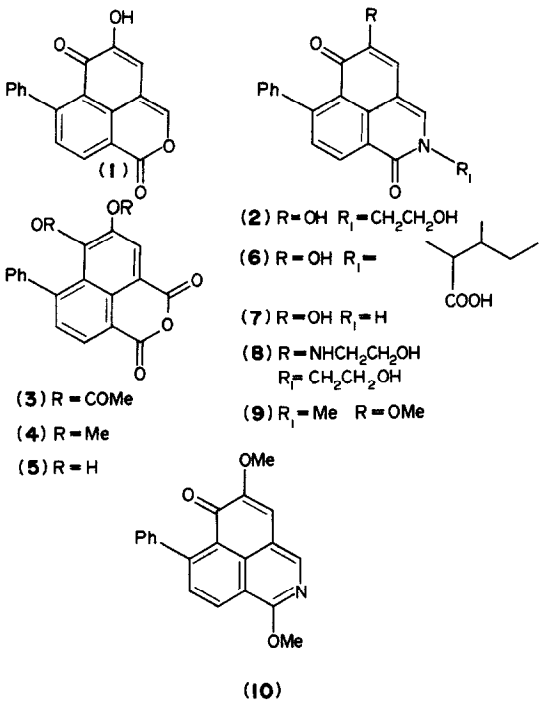
Abstract—Two new pigments have been isolated from flowers of *Lachnanthes tinctoria*. *N*-(1-carboxy-2-methylbutyl)-lachnanthopyridone is an oxygenated 9-phenylphenalenone with the nitrogen of an isoleucine group at position 5 of the ring system. 3,4-Dihydroxy-5-phenylnaphthalic anhydride, presumably derived from the *in vivo* oxidation of a 9-phenylphenalenone, has also been isolated. The synthesis of three 5-aza-9-phenylphenalenones is described.

Lachnanthes tinctoria Ell. Red Root, has already yielded a variety of colorful pigments. Many closely related 9-phenylphenalenone pigments, and several naphthalides and anhydrides, which are thought to be products of oxidative degradation [1,2], have been identified in the roots [2], seed capsules [3], and methylated extract of the plant [4]. Lachnanthopyrone (**1**) and *N*-(2-hydroxyethyl)-lachnanthopyridone (**2**) were isolated from an acetone extract of the inconspicuous yellow flowers of the plant [5]. We now wish to report the isolation of additional phenalenone pigments from the same source.

Silica gel chromatography of the acetone extract of the flowers yielded a yellow crystalline compound $C_{18}H_{10}O_5$. It produced a green color with $FeCl_3$ and showed IR absorptions at 3448 and 3175 cm^{-1}. These results, and the presence of a signal in the NMR spectrum at δ 3.70, which integrated for two protons and was exchangeable with D_2O, suggested that the compound was phenolic. Also evident in the NMR spectrum were a 5 proton singlet at δ 7.40 (assigned to an unsubstituted phenyl ring), and an AB quartet (J 7.6 Hz) at δ 8.40 and 7.50; of which the resonance at lower field is attributed to a proton deshielded by a carbonyl in a *peri* relationship to it.

Strong IR absorptions at 1770 and 1695 cm^{-1} indicated that the compound was an anhydride. Treatment of the compound with acetic anhydride in pyridine yielded a diacetate (**3**), $C_{22}H_{14}O_7$, whose NMR spectrum showed one of the acetoxy resonances at δ 1.43, significantly upfield from the other at δ 2.30. This shielding can be attributed to a phenyl group in a position *peri* to the acetoxy groups, and thus established the relative location of one of hydroxyls. The evidence so far described suggested a dihydroxy-5-phenylnaphthalic anhydride. A bright yellow dimethyl ether (**4**) was prepared using ethereal diazomethane. Its NMR spectrum again showed one of the methoxyl resonance (δ 3.40) shielded with respect to the other (δ 4.02) by the *peri* phenyl ring. The ether proved to be identical to a compound previously isolated by Cooke [6] during the structure proof of haemocorin aglycone, and as an oxidation product of methylated lachnanthopyrone (**1**) [5]. Structure (**5**) for the new pigment is in full agreement with all the spectral data. The methylated derivative (**4**) was also prepared by Cooke [4] as a derivative of 3-methoxy-4-hydroxy-5-phenylnaphthalic anhydride, isolated from *L. tinctoria* after prior methylation; **5** is clearly the natural product from which the 3-methyl compound was derived.



* [Part 6 of the series "Pigments of *L. tinctoria* Ell." Part 5. See ref. [2].

Further chromatography of the flower extract resulted in the isolation of a more polar bright orange pigment, $C_{24}H_{21}NO_5$. It proved readily soluble in aqueous $NaHCO_3$, and the IR spectrum showed bands characteristic of an amino acid [7], 2700–2200 (Broad) and 1738 cm^{-1}. Fischer esterification yielded a phenolic ($FeCl_3$) methyl ester which could not readily be crystallized. The exchangeable two proton resonance seen in the NMR spectrum of the compound was thus assigned to a phenolic and a carboxylic hydroxyl. The functionality was confirmed by the formation of a *p*-nitrobenzoate of the methyl ester. The mass spectrum of the new pigment contained a strong peak at m/e 288 similar to that seen in the MS of (**2**) [5], suggesting the presence of the ring system of lachnanthopyridone (**7**). The MS also contained the $(M-1)^+$ peak characteristic of 9-phenylphenalenones. The presence of the lachnanthopyridone chromophore was confirmed by the superimposability of the UV spectrum of the new compound and that of (**2**). The NMR spectrum of the new pigment showed aromatic and olefinic resonances very similar to those of (**2**), namely, an AB quartet at δ 8.70 and 7.75, attributable to protons in the 7 and 8 positions respectively of a lachnanthopyridone nucleus, a 5 H resonance at δ 7.35 due to a phenyl substituent at position 9, and two other olefinic proton resonances at δ 8.15 and 7.10. The NMR spectrum also contained upfield resonances suggestive of an isoleucine skeleton [8]. The presence of this amino acid moiety in the compound was confirmed by vigorous acid hydrolysis and paper chromatography. That (**6**) is the correct structural formula for the new pigment was further demonstrated by its synthesis from lachnanthopyrone (**1**) and L-isoleucine. The physical and spectral data for the natural and synthetic compounds are identical.

N-(2-hydroxyethyl)-lachnanthopyridone [5] was also synthesized from lachnanthopyrone and monoethanolamine. Again, the physical and spectral data for the synthetic and natural compounds are identical. When a threefold excess of ethanolamine was used a purple nonphenolic (no reaction with $FeCl_3$ or CH_2N_2) product, $C_{22}H_{20}N_2O_4$, was isolated. The IR spectrum: 1626 (pyridone CO), and 1612 (H bonded CO) was very similar to that of **2**; and the NMR spectrum contained resonances attributed to the ring protons at C-3 and C-4. The same compound was obtained by treating **2** with ethanolamine. These data indicate **8** as the probable structure of the product. Reaction of **1** with slight excess of ammonia gave the parent isocarbostyril, lachnanthopyridone (**7**) as a pale orange solid. All the spectroscopic data are in agreement with the structure; however the compound melted over a wide temperature range and no satisfactory combustion analysis could be obtained.

When lachnanthopyridone (**7**) was treated with an excess of etherial diazomethane, two isomeric dimethylated products were obtained; these were separated using Si gel PLC. The product of lower R_f yielded yellow crystals, mp 220–222°; its IR spectrum showed carbonyl bands similar to those of *N*-(2-hydroxyethyl)-lachnanthopyridone (**2**) and its methyl ether [5]. The aromatic resonances in the NMR spectrum were similar to those seen in the spectra of (**2**) and its derivatives, and the chemical shift of the second methyl resonance at δ 3.55 was in agreement with the *N*-methyl resonance seen in the spectrum of doryanine [9], an alkaloid containing a *N*-methylpyridone ring. The compound is thus assigned structure **9**: *N*-methyl-2-methoxylachnanthopyridone. The compound of higher R_f yielded orange crystals, mp 199–201°. The carbonyl region of the IR spectrum was not indicative of the presence of a pyridone ring. The NMR spectrum contained resonances indicative of the 9-phenyl substituent and the protons at C-7 and 8; however, the resonances at δ 8.12 (1H) and 4.15 (3H) were unlike those in the spectrum of **9** and were better accommodated by protons similar to H-6 and the 2-methoxy protons of 2-methoxypyridine [10]. It has been reported [11] that treatment of α-pyridone with diazomethane give both *N*- and *O*- methylation to yield *N*-methyl-α-pyridone and 2-methoxypyridine. This precedent, and the spectral evidence suggest that the less polar compound is 2,6-dimethoxylachnanthopyridine (**10**).

Closely associated with **6** and having an almost identical UV spectrum was a mixture of two additional pigments which could not be separated from each other by the chromatographic techniques available to us. Hydrolysis of the pigment mixture and paper chromatography indicated the presence of leucine and valine, suggesting that the plant contains analogs of **6** but derived from leucine and valine. We feel that the biosynthesis of *N*-(1-carboxy-2-methylbutyl)-lachnanthopyridone (**6**) may well parallel the *in vitro* synthesis by the incorporation of an intact molecule of isoleucine into lachnanthopyrone (**1**) and it seems likely that *N*-(2-hydroxyethyl)-lachnanthopyridone (**2**) results from a similar incorporation of serine, followed by decarboxylation. We plan to examine the flower extracts for a carboxylated serine derivative, and the decarboxylated analog of **6**.

EXPERIMENTAL

General. Mp's were determined with a Kofler hot stage apparatus and are corrected. NMR spectra were run in $CDCl_3$ unless otherwise noted. Samples for IR spectra were in the form of KBr discs, and MeOH was the solvent for UV spectra.

Isolation of the flower pigments. Flowers were exhaustively extracted with Me_2CO in a Waring blender. Filtration followed by evaporation under red pres yielded an aq residue which was extracted with $CHCl_3$. $CHCl_3$ soln was dried and concentrated. The concentrate was chromatographed over Si gel with C_6H_6–EtOAc. The new pigments eluted together, and further Si gel–$CHCl_3$ chromatography yielded pure (**5**) described below, as well as a mixed fraction which was further resolved using a dry packed polyamide column with $CHCl_3$ as elutant.

N-(1-*carboxy*-2-*methylbutyl*)-*lachnanthopyridone* (**6**). Further purification of the first material to elute from the polyamide column was achieved by chromatography on a Si gel column (made to activity grade V with 0.1M citrate–Pi buffer) with C_6H_6–EtOAc (19:1), and with subsequent PLC using plates prepared with a pH 3.6 citrate–Pi buffered slurry. Recrystallization from C_6H_6 yielded rosettes of orange crystals mp 218–219°. Found: M^+ 403.1410 (100), 288 (100). $C_{24}H_{21}NO_5$ requires 403.1419. ν_{max}: 3380 (H bonded phenol), 2700–2200, 1738 (amino acid), 1620 (2-hydroxyphenalenone), 1672 (σ lactam) cm^{-1}. λ_{max}: 240, 267, and 325 nm (log ϵ 4.44, 3.12, and 4.03); NMR (Me_2CO d_6): δ 8.70 (*d*, *J* 8 Hz; 1H), 7.55(*d*, *J* 8 Hz; 1H), 8.15(*s*, 1H), 7.35(*s*, 5H), 7.10(s, 1H), 5.55(*d*, *J* 10 Hz; 1H; collapses to s with decoupling at δ 2.25), 2.25(*m*, 1H), 2.10(*m*, 2H), 1.30–0.90(*m*, 6H). The red solution of (**6**) in NaOH was slowly changed to yellow by dithionite, and the compound was readily extracted from EtOAc by 5% $NaHCO_3$.

N-(1-*carbomethoxy*-2-*methylbutyl*)-*lachnanthopyridone*-p-*nitrobenzoate*. The methyl ester, formed by treatment with CH_3OH and H_2SO_4 in C_6H_6, could not be satisfactorily crystallized, it was however homogeneous by TLC (buffered Si

gel, EtOAc–C_6H_6, 1:2). Found M^+ 417.151. ($C_{25}H_{23}NO_5$ requires 417.157). ν_{max}: 1741 (ester CO), 1668, 1611 cm^{-1}. The ester was treated with *p*-nitrobenzoyl chloride in pyridine to yield the *p*-nitrobenzoate methyl ester, mp 199–200°, from EtOH–H_2O. Found: C, 67.44; H, 4.68; N, 4.82. $C_{32}H_{26}N_2O_8$ requires C, 67.82; H, 4.63; N, 4.95. ν_{max} 1750, 1680, 1635, 1610, 1530, 1262 cm^{-1} NMR: δ 8.70(*d*, *J* 8 Hz, 1H), 8.30(*s*, 4H), 8.00(*s*, 1H), 7.58(*d*, *J*8 Hz, 1H), 7.47(*s*, 1H), 7.39(*s*, 5H), 5.75(*d*, *J*10 Hz, 1H), 3.82(*s*, 3H), 2.00(*m*, 2H), 1.10(*m*, 8H). Found M^+ 566.1694. $C_{32}H_{26}N_2O_8$ requires 566.1685.

Acid hydrolysis of N-(1-*carboxy*-2-*methylbutyl*)-*lachnanthopyridone*. Compound (**6**) (2 mg) was heated with 2 ml 6M HCl in a sealed glass tube in an autoclave (112°) for 48 hr. Hydrolyzate was extracted with EtOAc, and the aq. phase repeatedly evaporated after addition of small vols H_2O to remove HCl. Two-D PC (Whatman No. 1; *t*-BuOH–formic acid–H_2O; 250:3.6:106, *n*-BuOH–HOAc, H_2O; 4:1:5) alone or with co-spotting with L-ile yielded only 1 ninhydrin positive spot.

Acid hydrolysis of analogs of (**6**). These pigments showed a very slightly lower R_f than (**6**) and were separated from it by development of buffered Si gel columns or through multiple development of the buffered PLC system described above. The constituents of the mixture could not be separated, but hydrolysis as described for (**6**), and PC, co-spotting with leu and val, established that these amino acids were hydrolysis products of the mixture.

3,4-*Dihydroxy*-5-*phenylnaphthalic anhydride* (**5**) eluted from the polyamide column after (**6**). Recrystallization from $CHCl_3$ gave yellow needles, mp 244–245°, which were sublimed (180°, 0.1 mm Hg). Found: C, 70.85; H, 3.54; M^+ 306,049. $C_{18}H_{10}O_5$ requires: C, 70.59; H, 4.29; M^+ 306,0528. ν_{max} 3448, 3175 (phenolic OHs), 1770 and 1695 cm^{-1} (anhydride); λ_{max} 266 and 345 nm (log ϵ 4.27 and 3.72); NMR (220 M Hz, Me_2CO d_6) δ 8.40(*d*, *J* 7.5 Hz, 1H), 8.27(*s*, 1H), 7.50(*d*, *J* 7.5 Hz, 1H), 7.40(*s*, 5H), 3.20(*s*, 2H, exchange with D_2O). The diacetate (**3**) crystallized from C_6H_6 as pale yellow needles, mp 193–194°. Found C, 67.79; H, 3.68, M^+ 390.0744. $C_{22}H_{14}O_7$ requires C, 67.69; H, 3.62, M^+ 390.0739. ν_{max}: 1786, 1745, 1600, 1587, 1020 cm^{-1}; λ_{max}: 239 and 310 nm (log ϵ 4.27 and 3.72) NMR: δ 8.56(*d*, 8 Hz, 1H), 7.60(*d*, 8 Hz, 1H), 7.45(*s*, 5H), 2.30(*s*, 3H), 1.43(*s*, 3H). The dimethyl ether (**4**) was obtained as bright yellow crystals, mp 138–140° from C_6H_6 (lit. [6] 157° from EtOH). Found M^+ 334.085. $C_{20}H_{14}O_5$ requires 334.084. ν_{max} 1765, 1734 and 1570 cm^{-1}; NMR: δ 8.43(*d*, 8 Hz, 1H), 8.41 (*s*, 1H), 7.55(*d*, 8 Hz, 1H), 7.41(*s*, 5H), 4.02(*s*, 3H), 3.40(*s*, 3H).

N-(1-*carboxy*-2-*methylbutyl*)-*lachnanthopyridone*. Lachnanthopyrone (50 mg, 0.17 *m* mol) in MeOH (100 ml) and isoleucine (22.6 mg, 0.17 m mol) were stirred at room temp overnight. MeOH was evaporated and the residue taken up in $CHCl_3$. The product was then extracted into 5% $NaHCO_3$. Acidification, followed by extraction back into $CHCl_3$ and recrystallization from C_6H_6, gave in low yield a product identical to the natural product by TLC, mp, IR and NMR. Found: C, 71.22; H, 5.37; N, 3.54. $C_{24}H_{21}NO_5$ requires C, 71.44; H, 5.25; N, 3.47. The $NaHCO_3$ washed $CHCl_3$ contained an unidentified non acidic yellow product, R_f 0.3 (buffered Si gel, EtOAc–C_6H_6, 1:3).

N-(2-*hydroxyethyl*)-*lachnanthopyridone* (**3**). Addition of monoethanolamine (0.02 g, 0.33 m mol, in MeOH) to lachnanthopyrone (100 mg, 0.33 mmol, in 100 ml MeOH) resulted in an immediate color change from yellow to orange. After 7 days of stirring the solvent was evaporated and the residue, after recrystallization from EtOAc, yielded orange crystals identical in mp and IR to the natural product. Reaction with an excess of ethanolamine gave (**8**). mp 234–239° from MeOH. Found M^+ 376.1426 $C_{22}H_{20}N_2O_4$ requires 376.1423. ν_{max}: 1626 and 1612 cm^{-1}. NMR: δ 8.60(*d* *J*8 Hz), 7.80(*s*), 7.50(*d* 8 Hz), 7.40(*m*), 6.90(*s*), 4.10(*m*), 3.80(*m*).

Lachnanthopyridone (**7**). An excess of NH_3 (approx. 0.3 ml conc. soln) was added to lachnanthopyrone (100 mg, 0.33 mmol), in MeOH (100 ml). An immediate color change from yellow to brown was evident. The mixture was stirred for 7 days, the solvent was evaporated and the residue treated with dil HCl. The product was extracted into EtOAc and the soln was dried. Chromatography of the product over polyamide with MeOH as elutant followed by recrystallization gave poor crystals, mp 338–342°, from acetone. This material was sublimed at 300° and 0.1 mm Hg. No satisfactory combustion analysis could be obtained and the material was not suitable for peak matching. ν_{max}: 3440, 1660, 1610, 1290 cm^{-1} NMR (220 MHz, d_6DMSO): δ 8.93(*s*, 1H, exch, with D_2O), 8.55(*d*, 8 Hz, 1H), 7.90(*s*, 1H), 7.54(*d*, 8 Hz, 1H), 7.37(*s*, 5H), 7.04(*s*, 1H).

N-*methyl*-2-*methoxylachnanthopyridone*(**9**) *and* 2,6-*dimethoxylachnanthopyridine* (**10**) were prepared by treating sublimed lachnanthopyridone (**7**) with an excess of ethereal diazomethane. The compounds were separated using Si gel PLC with EtOAc–MeOH (19:1). Recrystallization of the less mobile product **9**, from C_6H_6–hexane yielded yellow crystals, mp 220–222°. Found: M^+ 317.1071. $C_{20}H_{15}NO_3$ requires 317.1052. ν_{max}: 1667, 1645, 1621, and 1597 cm^{-1}. NMR: 8.73(*d*, 8 Hz; 1H), 7.43(*d*, 8 Hz; 1H), 7.33(*s*, 1H), 7.25(*s*, 5H), 6.47(*s*, 1H); 3.68(*s*, 3H), 3.55(*s*, 3H). Recrystallization of the product with higher R_f (**10**) from hexane yielded orange crystals, mp 199–201°. Found: M^+ 317.1033. $C_{20}H_{15}NO_3$ requires 317.1052. ν_{max}: 1645, 1629, 1592, 1459, and 1250 cm^{-1}. NMR: δ 8.47(*d*, 8 Hz, 1H), 8.12(*s*, 1H), 7.54(*d*, 8Hz, 1H), 7.30(*s*, 5H), 6.75(*s*, 1H), 4.15(*s*, 3H), 3.8(*s*, 3H).

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