INVESTIGATION OF *ERYTHRINA* SPP. IX. CHEMICAL CONSTITUENTS OF *ERYTHRINA STRICTA* BARK[1]

HARKISHAN SINGH, AMRIK SINGH CHAWLA, VIJAY K. KAPOOR and NAROTAM KUMAR

Department of Pharmaceutical Sciences, Panjab University, Chandigarh 160014, India

and

D. M. PIATAK and W. NOWICKI

Department of Chemistry, Northern Illinois University, Dekalb, Illinois 60115

ABSTRACT.—The petroleum ether extractive of the bark of *Erythrina stricta* was resolved into various non-nitrogenous fractions which consisted of *n*-alkanes, fatty esters, *n*-alkanols, alkyl ferulates, fatty acids, sitosterol and stigmasterol.

The ethanol extractive yielded a coumarin entity, identified as 7-methoxy-8-(15-hydroxypentadecyl)-coumarin (**1**); two chloroform-soluble bases, erysovine (**2**) and erysodine (**3**); and a water-soluble base characterized as hypaphorine.

Erythrina stricta Roxb. has been used in the indigenous system of medicine for various ailments (1). Continuing with our research program on the investigation of Indian *Erythrina* species for alkaloidal and non-alkaloidal constituents (2–9), the bark of *E. stricta* has now been examined. This bark had not been studied previously. However, from the seeds Games *et al.* (10) have reported the occurrence of erythraline, erysodine, erythrinine, erysopine, 11-hydroxyerysodine and 11-hydroxyerysovine as determined by gc-ms studies.

RESULTS AND DISCUSSION

The petroleum ether extractive of the powdered bark, on chromatography over alumina, yielded gummy solids, a partial purification of which was attempted by crystallization. Fractions A–G were thus obtained.

The physical characters and a study of the ir spectra indicated fraction A to be a mixture of *n*-alkanes, fraction B consisted of fatty esters, fractions C and E consisted of fatty alcohols, and fraction D to be a mixture of fatty alcohols and sterols. The ir and uv spectral data and the nmr spectral characteristics suggested that fraction F was an alkyl ferulate. Fraction G was found to be composed of *n*-aliphatic fatty acids on the basis of spectral characteristics.

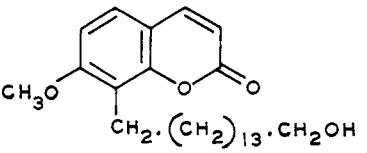
The alkanes present in fraction A were shown by glc to be predominantly heptacosane, octadecane and tritriacontane. The fatty esters from fraction B had C_{29} and C_{19} as the major components. The fatty alcohols obtained from fractions C, D and E were shown by glc to consist of hexatriacontanol and dotriacontanol; hexatriacontanol, octatriacontanol and dotriacontanol; and pentatriacontanol and heptatriacontanol as the major components, respectively. Fraction D, in addition to fatty alcohols, contained sitosterol and stigmasterol. Fraction F was saponified and separated into alcohols and the acid, characterized as ferulic acid. The fatty alcohols from fraction F had dotriacontanol as the major component, and hexatriacontanol and pentatriacontanol were present in appreciable amounts. The wax alcohol esters appear to be common in tree barks (11). The wax acids comprising fraction G were converted to methyl esters. Glc showed the methyl esters to be predominantly those of hexacosanoic, octacosanoic, and tetracosanoic acids.

The marc left after separation of the petroleum ether extractive was exhausted

[1]For previous paper, see reference (9).

with ethanol. The ethanol extract after being kept at room temperature for 2–3 days deposited a granular solid which, on chromatography over alumina, yielded two entities labelled as fractions H and I. Fraction H was identified as a fatty alcohol.

The ir spectrum of fraction I gave bands at 3460 cm^{-1} (O–H), 1736 cm^{-1} (α-pyrone moiety), 1655 cm^{-1} (C=C), 1613, 1527 and 1468 cm^{-1} (aromatic) and 725 cm^{-1} [$-(CH_2)_x-$]. The uv spectrum showed λ max (MeOH) at 325 nm (ϵ 10,670) and 235 nm (ϵ 6834) due to α-pyrone and benzenoid moieties (12, 13). The nmr spectrum showed signals at δ 6.26 (d, $J=15$ Hz, 1H) and 7.59 ($J=15$ Hz, 1H) arising from C-3 and C-4 protons, respectively. The aromatic proton signals appeared at δ 7.06 (d, $J=8$ Hz, 1H) and 6.88 (d, $J=8$ Hz, 1H). The coupling constant indicates that these two protons are located ortho to each other. There were also present a triplet centered at δ 4.18 ($-CH_2O$), a sharp singlet at δ 3.91 (aryl- OCH_3), and signals at δ 1.65 (2H) and δ 1.26 (27H). The mass spectrum gave a parent ion (M^+) peak at *m/e* 402. On the basis of these results, fraction I appears to have structure **1**. This is the first time that a coumarin has been isolated from the *Erythrina* genus and is a new entity.

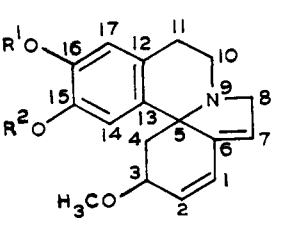


(1)

The ethanol extract, after the removal of the granular solid, on usual work up yielded two chloroform-soluble bases (fractions J and K) and a water-soluble base.

The nmr spectrum (60 MHz) of fraction J in $CDCl_3$ showed two aromatic proton signals at δ 6.85 (s, 1H, 17–H) and 6.63 (s, 1H, 14–H). The 1–H, 2–H and 7–H protons appeared as diffuse signals at δ 6.0, 6.45 and 5.67, respectively. Two methoxyl group singlets appeared at δ 3.33 and 3.87. The uv spectrum showed λ max (MeOH) at 283 nm (log ϵ 3.6) and 228 nm (log ϵ 4.3). The mass spectrum of the base showed significant peaks at *m/e* 299 (M^+, 39%), 284 (M^+-CH_3, 41%), and 268 (M^+-OCH_3, base peak). On the basis of these results and elemental composition data, the isolated base was characterized as erysovine (**2**).

The nmr spectrum (90 MHz) of fraction K gave signals at δ 6.77 (s, 1H, 14–H), 6.67 (s, 1H, 17–H), 6.55 (dd, $J=10$ Hz, 2.5 Hz, 1H, 2–H), 5.95 (d, $J=10$ Hz, 1H), 5.70 (s, 1H, 7–H), 3.30 (s, 3H, CH–OCH_3) and 3.75 (s, 3H, aryl OCH_3). The



(2) $R^1=CH_3$, $R^2=H$

(3) $R^1=H$, $R^2=CH_3$

uv spectrum showed λ max (MeOH) at 283 nm (log ε 3.7) and 228 nm (log ε 4.3). The mass spectrum indicated peaks at *m/e* 299 (M^+, 72%), 284 (M^+–CH_3, 47%) and 268 (M^+–OCH, base peak). These spectral results spotted the fraction to be erysodine (**3**), the identity of which was finally established through comparison of the infrared spectra which were superimposable.

The water-soluble base was identified as hypaphorine.

EXPERIMENTAL

PLANT MATERIAL.—The plant material used in this investigation was procured through Mr. Mohammad Kutty, Cheruthuruthy (Kerala), and was reduced to a moderately coarse powder.

EXTRACTION OF PLANT MATERIAL.—The bark powder (2 x 1.5 kg) was extracted with petroleum ether in a Soxhlet unit to obtain a residue (26 g). The marc left after extraction with petroleum ether was extracted with ethanol. The ethanol extract was allowed to stand at room temperature for 2–3 days. A granular solid residue (4.2 g) separated, which was removed by filtration, and the ethanol extract (120 g) was processed for the isolation of alkaloids.

PETROLEUM ETHER EXTRACT FRACTIONS.—The extract (26 g) obtained above was resolved on an alumina column [Neutral, BDH (India), according to Brockmann, 400 g]. Elution was started with petroleum ether-benzene (2:1). The first few eluates (4 x 150 ml) gave a residue (2.3 g) liquid at room temperature (fraction A), and the subsequent elutions gave a waxy residue (650 mg, fraction B). The first few eluates obtained with benzene-chloroform (1:1, 6 x 150 ml) yielded a residue (0.63 g), mp 70–73°, (fraction C). The later eluates (6 x 150 ml) afforded a waxy residue (5.1 g) which was crystallized from petroleum ether, mp 135–138°, (fraction D). The mother liquor separated after the crystallization of fraction D was concentrated and allowed to stand at room temperature. An amorphous residue, mp 76–80°, was obtained (fraction E). The residue obtained on elution with chloroform (8 x 150 ml), and chloroform-methanol (49:1, 6 x 150 ml; 19:1, 8 x 150 ml) were found to be identical and were mixed together. On crystallization from petroleum ether, the combined fractions gave a solid residue (1.4 g), mp 67–70°, (fraction F). Finally, elution was carried out with chloroform-acetic acid (49:1, 6 x 150 ml). The residue (6.8 g) obtained was refluxed with petroleum ether. The petroleum ether-soluble part was crystallized (1 g), mp 75–77°, (fraction G).

FRACTIONATION OF THE GRANULAR RESIDUE.—The granular residue (4.2 g) was taken up in benzene (150 ml) and resolved over alumina (200 g). Elutions with benzene-chloroform (1:1, 10 x 100 ml) afforded a waxy residue (0.5 g) which, on repeated crystallizations from acetone, afforded an entity, mp 77–79°, (fraction H). The eluates obtained on elution with chloroform-methanol (19:1, 6 x 100 ml; 9:1, 4 x 100 ml; 4:1, 4 x 100 ml) were found to be identical and were mixed together (0.2 g). This, on repeated crystallizations from acetone-petroleum ether (60–80°), gave an entity, mp 80–82° (fraction I).

STUDY OF DIFFERENT FRACTIONS

Fraction A-: Gas chromatography of fraction A showed it to be a mixture of *n*-alkanes consisting of C-14 (3.3%), C-16 (3.0%), C-18 (11.3%), C-20 (1%), C-21 (1%), C-22 (1%), C-23 (2.9%), C-24 (4%), C-25 (6.1%), C-26 (7%), C-27 (13.1%), C-28 (8.6%), C-29 (8.5%), C-30 (9.2%), C-31 (8.9%) and C-33 (11.3%).[2]

Fraction B-: Gas chromatography of fraction B indicated peaks corresponding to fatty esters, C-11 (1%), C-14 (2.6%), C-15 (1%), C-16 (5.5%), C-17 (8.2%), C-18 (4.5%), C-19 (10.9%), C-20 (5.9%), C-21 (8.8%), C-22 (4.0%), C-23 (7.8%), C-24 (7.0%), C-26 (6.0%), C-27 (2.7%), C-27 (4.0%), C-28 (4.9%) and C-29 (11.5%).

Fraction C-: Gas chromatography of fraction C showed it to be a mixture of *n*-alkanols consisting of C-20 (3.0%), C-22 (2.7%), C-24 (6.6%), C-26 (10.1%), C-28 (11.7%), C-30 (3.5%), C-32 (23.9%), C-34 (4.1%), C-35 (1.7%), and C-36 (32.7%).

Fraction D-: Gas chromatography revealed it to be a mixture of *n*-alcohols corresponding to C-24 (1%), C-28 (2.4%), C-30 (1%), C-31 (1%), C-32 (21%), C-36 (38.3%), C-38 (23.1%), C-39 (4.2%), and sitosterol (4.1%), stigmasterol (5%). Similarly *fraction E* was found to be a mixture of fatty alcohols consisting of C-27 (1.5%), C-29 (2.3%), C-31 (3.6%), C-32 (5.6%), C-33 (6.8%), C-34 (2.3%), C-35 (51.7%), C-37 (16.8%) and C-39 (5.5%).

Fraction F-: Spectral examination of fraction F showed the following: ir, ν max (KBr) 3390

[2]Glc analyses were performed on a Varian-Aerograph Series 2700 instrument with a flame ionization detector. It was coupled to an Autolab System IV computing integrator. The hydrocarbon fractions were determined on a ⅛ inch by 5 ft. column of OV-101 (1.5%) on 100/120 HP Chrom G with a temperature program of 4°/min. from 95–240°. The *n*-alkanols were analyzed isothermally on the same column at 240°. Compound identifications were accomplished by coinjection of and correlation with authentic standards (Applied Science Lab.) and extrapolation of retention times. Some signals were verified by combined gc-ms analysis.

cm^{-1} (O−H), 1724 cm^{-1} (C=O), 1640 cm^{-1} (C=C), 1603 and 1530 cm^{-1} (aromatic), 1274 cm^{-1} (C−O), and 730 and 720 cm^{-1} [$-(CH_2)_x-$]; uv, λ max ($E^{1\,cm}_{1\%}$) 217 nm (430.7), 232 nm (342.3), 295 nm (338.4) and 324 nm (426.9); nmr, δ 7.61 (d, J=15 Hz, phenyl. CH=), 6.27 (d, J=15 Hz, $=CH-\overset{O}{\overset{\|}{C}}-OR$), 7.01 (s, 2H), 6.94 (s, 1H), 4.20 (m, CH_2O-), 3.93 (s, $-CH_3O$), 1.25 [s, $-(CH_2)_x-$] and 0.91 (m, $-CH_2-CH_3$). The nmr was identical to that of the wax alcohol ferulate.

The fraction was saponified by refluxing in 2N alcoholic KOH for 4 hr under nitrogen, concentrated, diluted with water, and extracted with ether; the residue was shown to be wax alcohols. Glc analysis showed the presence of C-22 (3.3%), C-24 (9.5%), C-26 (4.8%), C-28 (5.6%), C-31 (11.7%), C-32 (33.2%), C-34 (2.1%), C-35 (14.6%) and C-36 (15.1%).

The aqueous layer, after extraction of alcohols, was acidified and extracted with ether. The extracted acid was identified as ferulic acid by ir, uv and paper chromatography on Whatman No. 1 with the solvent benzene-acetic acid and water (125:72:sat), and had the same color reactions (14).

Fraction G-: Spectral examination of fraction G showed the following: ir, ν max (KBr), 3440 cm^{-1} (O−H), 1712 cm^{-1} (C=O), and 730 and 720 cm^{-1} [$-(CH_2)_x-$]; nmr, identical to that of fatty acids. The fatty acids were esterified with CH_2N_2 and analyzed by glc to consist of C-16 (1%), C-18 (1.2%), C-20 (3.1%), C-21 (1.5%), C-22 (10.4%), C-23 (4.6%), C-24 (12.8%), C-25 (6.8%), C-26 (28.9%), C-27 (3.6%) and C-28 (23.8%).[3] A semi-log plot of the isothermal glc analysis confirmed the identifications obtained by temperature programming.

Fraction H-: The spectral characteristics showed this fraction to be a fatty alcohol.

Fraction I-: The general characters and a study of the spectra indicated that this fraction is a substituted coumarin (**1**). Tlc on silica gel with benzene-ether (9:1) and benzene-chloroform (3:1) gave a single spot.

ISOLATION AND IDENTIFICATION OF THE ALKALOIDS.—The ethanol extractive (120 g) was repeatedly treated with 2% w/v sulphuric acid. The acid solution was washed with chloroform (6 x 100 ml), basified with dilute ammonia solution (340 ml) and extracted with chloroform (9 x 200 ml). When chloroform extract was washed with water, dried (Na_2SO_4) and evaporated the alkaloidal residue (1.5 g) was obtained. It was chromatographed over alumina (75 g). Elutions with benzene-chloroform (1:1, 4 x 50 ml; 2:3, 8 x 50 ml) gave a waxy residue (0.3 g) which was crystallized from acetone-petroleum ether (60–80°) to obtain a base, mp 167–169°, undepressed on admixture with authentic erysovine (**2**). Co-tlc ($CHCl_3$–CH_3OH, 9:1) with erysovine showed a single spot. *Anal*: Calcd. for $C_{18}H_{21}NO_3$: C, 72.22; H, 7.07; N, 4.68. Found: 72.37; H, 7.23; N, 4.71.

Elutions with chloroform (4 x 50 ml) yielded a sticky mass (0.1 g) which was crystallized from acetone to obtain a base, mp 200–202°, identified as erysodine (**3**).

The aqueous layer left above was acidified and the bases precipitated with Dragendorff's reagent. The separated precipitate was decomposed with silver carbonate and processed to obtain a crystalline entity, mp 260–262°, and analyzed for elemental composition $C_{14}H_{18}N_2O_2$.

Anal: Calcd. for $C_{14}H_{18}N_2O_2$: C, 68.27; H, 7.37; N, 11.37. Found: C, 68.52; H, 7.52; N, 11.45.

The base showed no depression in melting point on admixture with authentic hypaphorine, and the infrared spectra of both were identical.

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[3]The methylated fatty acids were analyzed on a ⅛ inch by 7 ft. column of 15% diethylene glycol succinate (DEGS) on 80/100 WAW by programming from 100–250° at 4°/min. The components were differentiated by comparison with standards.