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Structure Elucidation of Erythrosotidienone and Erythromotidienone — Two New Isoquinoline Alkaloids from *Erythrina variegata* Flowers†

SHIV K. SHARMA and H. MOHINDRA CHAWLA*

Department of Chemistry, Indian Institute of Technology, Hauz Khas, New Delhi-110 016

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Careful examination of the acetone extract of the *Erythrina variegata* flowers has revealed the presence of five compounds, three of which are identified as stigmasterol (1), cycloartenol (2) and erysotramidine (13). The remaining two compounds are new isoquinolines whose structures have been established on the basis of chemical correlation, derivatization and ir, ^{1}H nmr, ^{13}C nmr and mass spectral analysis. They are tentatively named as erythrosotidienone (4) and erythromotidienone (10). Cycloartenol (2) has been isolated for the first time from *Erythrina* species. The occurrence of the new compounds 4 and 10 is of interest from the biogenetic point of view.

Erythrina (Family-Leguiminosae, subfamily-Papilionaceae) is an important genus among the trees and shrubs consisting of about fifty species and is found in the tropic and subtropic zones of all parts of the world[1–3]. Various parts of some species such as *arbovescens*, *stigmoides*, *stricta*, *suberosa* and *variegata* are reported to possess pharmacological and medicinal properties[4–6]. These were usually attributed to their rich alkaloidal content which is normally considered as a reservoir of protein synthesis, as a protective material discouraging animal or insect attacks, as plant stimulants or regulators for growth, metabolism and reproduction, or as detoxicating agents for substances whose accumulation might otherwise cause damage to the plant[7,8].

Erythrina variegata is a tall ornamental tree, reaching about 10 m in height, and is widely distributed throughout the upper Gangetic plains of India, including Assam[9,10]. Various extracts of different parts of this plant find use in the indigeneous system of medicine and have been found antiasthmatic and a collyrium in ophthalmia[10-12]. The leaves of the plant are used as a diuretic and for relieving pain in joints. A thorough literature survey indicates that leaves, seeds, stem bark and root bark of *E. variegata* have been examined[1,12–16] for their chemical components but very little work seems to have been reported on its flowers which was taken up in the present study.

In this paper, we wish to report an account of the isolation and structural elucidation of five compounds (designated as A_1-A_5) from the acetone extract of flowers of *E. variegata* two of which are new natural products.

Identification of compounds :

The concentrated acetone extract of the shade-dried flowers of *Erythrina variegata* on maceration with methanol afforded the methanol-soluble and methanol-insoluble fractions. The methanol-insoluble fraction when subjected to column chromatography over silica gel, gave two compounds, designated A_1 and A_2, while the methanol-soluble fraction, when chromatographed over neutral alumina, afforded three compounds, designated A_3, A_4 and A_5.

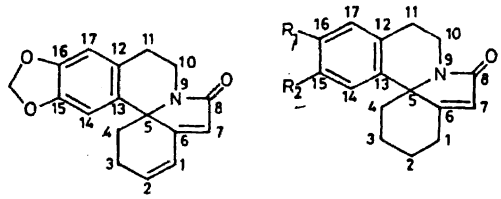
Compound A_1, crystallized as white needles, m.p. 168°, was identified as stigmasterol (1) by colour reaction, ir, ^{1}H nmr and mass spectral fragmentation pattern, and by preparation of the derived acetate[17,18].

Compound A_2 was crystallized from benzene-chloroform as white needles, m.p. 116°, and gave a positive Liebermann-Burchard test. From a detailed analysis of its ir, ^{1}H nmr and mass spectral fragmentation pattern, it was identified as cycloartenol (2), which was confirmed by its literature data[19,20]. This is the first report of the occurrence of this compound in *Erythrina* species.

Compound A_3 designated as erythrosotidienone, $C_{17}H_{15}NO_3$ (also, M^+ 281), neither gave any colour with $FeCl_3$ nor a positive reaction with Liebermann-Burchard reagent, which indicated the absence of a phenolic group and a steroidal or a triterpenoidal skeleton in the molecule respectively. Since it responded to a colour reaction with Meyers reagent, it was considered to be an alkaloid. Strong ir absorption at 1740 cm^{-1} suggested the presence of a five-membered lactam ring which is a common structural feature among *Erythrina* alkaloids, and this was confirmed by the ^{13}C nmr signal at δ 184.0. The other absorptions at 1610 and 1160 cm^{-1} reflected the presence of an unsaturation and an ether linkage respectively in the molecule. The ^{1}H nmr spectrum of compound A_3 showed the presence of a methylenedioxy group (δ 5.94, 2H, s) and three aromatic protons (δ 7.32, 7.23 and 6.62, 1H, s each, H-14, 17, 7 respectively). In the mass spectrum, the molecular ion peak at *m/z* 281 suffered two successive losses of 28 and 26 units to give respective intense fragments, at *m/z* 253 and 227. Since its ^{1}H nmr showed a total number of 15 protons (also confirmed by MS), there should be one more double bond

†Dedicated to Professor Sukh Dev on his 75th. birthday.

present, which could be accommodated in any one of the four structures **3**-**6**.



3 : Δ^{10} , **5** : Δ^{2}

4 : Δ^{1} , **6** : Δ^{3}

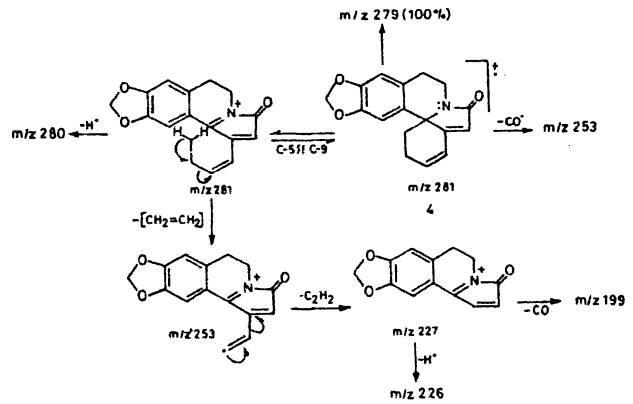
7 : Δ^{3} , **9** : Δ^{2}

8 : Δ^{10} , **10** : Δ^{1}

for 7–10 : R_1/R_2 = H/OMe

The structures **3** and **5** were rejected as only one doublet and a multiplet in the low field, i.e. at δ 6.02 and 5.72 respectively, were observed in the ^{1}H nmr spectrum of compound A_4, whereas the requirements for the structure **3** should be two doublets and for structure **5** there should be two multiplets. On the other hand, both the structures **4** and **6** equally explained the above observation and ^{1}H nmr results could not resolve the constitution of A_3. The problem was solved by the mass spectral analysis . The observed successive loss of 28 and 26 m.u. from the M^+ are only possible for **4** as structure **6** should first lose an acetylene molecule to give the fragment at *m/z* 225, which was not observed.

The derived structure **4** of compound A_3 was compatible with the major mass spectral fragmentation pattern (Scheme 1) and the high resolution ^{1}H nmr spectral data (vide experimental). The proposed structure **4** was fully in agreement with its ^{13}C nmr (including SFORD). The tentative assignments have been shown in the experimental section.



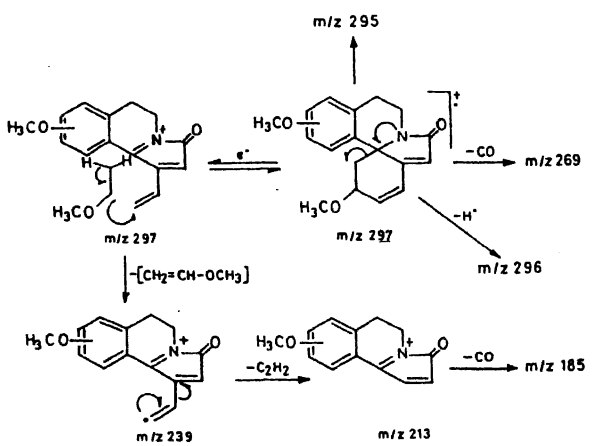
Scheme 1. Mass spectral fragmentation of erythrosotidienone (**4**). Except for M^+, only peaks with r.a. of 15% and above have been shown. The peak at *m/z* 81 remains unexplained.

Compound A_4 designated as erythromotidienone, $C_{18}H_{19}NO_3$, gave a positive Meyers reagent test and the DNP test for a ketonic functionality but did not respond to the $FeCl_3$ and Liebermann-Burchard reactions. It showed strong ir bands at 1760, 1600 and 1170 cm^{-1} which are suggestive of a five-membered lactam ring, an unsaturation and an ether linkage respectively, in the compound. The other physical and chemical properties of A_4 indicate that it has an *Erythrina*-type skeleton, similar to that of A_3.

In the ^{1}H nmr spectrum, A_4 exhibited signals for two methoxyls – one aryl (δ 3.86, s) and the other aliphatic (δ 3.36, s) – and those typical of an *Erythrina* alkaloid skeleton, viz. three aromatic protons (δ 7.13, 7.21, 7.32), three olefinic protons (δ 6.02, 6.34, 6.62) and seven aliphatic protons (δ 1.92–3.76, m).

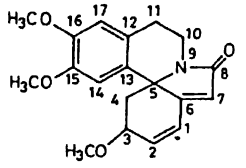
In the mass spectrum, compound A_4 showed peaks at *m/z* 297 (M^+), 296 (M-1), 295 (M-2), 269 (M-CO) and 266 (M-OCH_3). An intense fragment at *m/z* 239 could be ascribed to the loss of methyl vinyl ether molecule (a loss of 58 units) which revealed that the second methoxyl is attached to the C-3 position, as found in other *Erythrina* alkaloids[12–16]. To establish the position of double bond, four possible structures **7**-**10** were considered for compound A_3.

Structure **7** was rejected as only one singlet (δ6.62, 1H) was observed in its ^{1}H nmr spectrum as against the expected two singlets for it. The structures **8** and **9** were ruled out as there would be two doublets and a triplet respectively in the low field but we observed only a doublet and a double doublet in the ^{1}H nmr spectrum. This left structure **10** to be the likely structure for A_4. This was confirmed by analysis of the remaining peaks in the ^{1}H nmr spectrum, so the doublet (δ 6.34) and the double doublet (δ 6.02) could be ascribed to the C-1 and C-2 protons respectively. Structure **10** was also in agreement with its mass spectral fragmentation, as depicted in Scheme 2.



Scheme 2. Mass spectral fragmentation of erythrosotidienone (**10**).

The exact position of the methoxyl group, i.e. whether it is attached to C-15 (**11**) or C-16 (**12**) could not be ascertained due to insufficient amount of the sample which did not allow exe-cution of chemical reactions that might have shed light on the true structure for the compound.



13

Compound A_5 was identified as erysotramidine (**13**) by its MS data and was confirmed by comparison of its ir and ^1H nmr data reported in literature[21].

Though analogous synthetic compounds have been described in the literature. Erythrosotidienone (**4**) is the only naturally occurring compound among *Erythrina* alkaloids which does not have a methoxyl group in the C-3 position. At this stage, a comment on possible biogenesis of erythrosotidienone (**4**) is in order. Prelog *et al.*[22,23] and Koniuszy and Folkers[24] reported that an *Erythrina* alkaloid with a methoxyl functionality at C-3 incurs a loss of methanol to yield a compound which has a double bond between C-3 and C-4 on treatment with a mineral acid at room temperature. In some cases, the newly generated double bond has been determined to undergo an apo-rearrangement to give other isomers[25,26]. We believe that a similar pathway might be operative during the biogenesis of erythro-sotidienone via and intermediate **15** which can be formed from compound **14** (Scheme 3). The carbonyl group of **15** might undergo biological reduction to afford **16** which yields **17** and **4** via 1,7-hydrogen shift and prototopic tautomerism respectively. On the other hand, there is a possibility that **15** might directly lead to a product by reduction of a double bond between positions C-3 and C-4.

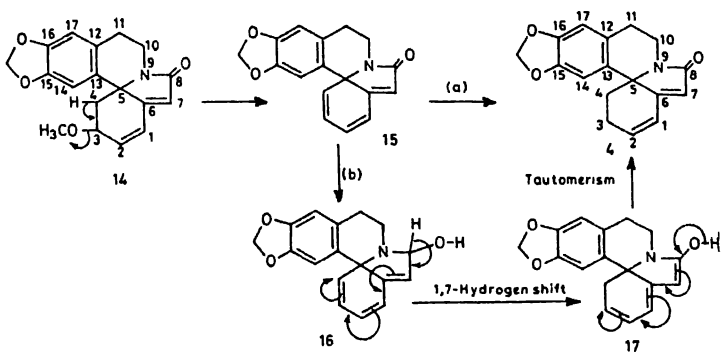
While the petroleum ether extract[27] revealed the presence of steroids, fatty acids and only one known alkaloid, the acetone extract seems to be rich in alkaloids. Cycloartenol (**2**) is being reported for the first time from an *Erythrina* species.

Experimental

All m.ps. were determined on an electric melting point apparatus (Adair Dutt) in open capillaries and are uncorrected. ^1H and ^{13}C nmr spectra were recorded in $CDCl_3$ on 99.50 MHz JOEL FT NMR spectrometer using TMS as the internal standard and chemical shifts are reported in δ scale. IR spectra were run on KBr discs on SP-1200 and 5DX-Nicolet FT IR spectrometers. Mass spectra were recorded on a JEOL JMS-D-300 spectrometer at 70 eV. The purity of the compounds were monitored by TLC performed on silica gel (B.D.H.) plates using iodine for visualizing the spots. Column chromatography was carried out by using silica gel (60–120 mesh) and neutral alumina. The organic solvent extracts were usually dried over anhydrous $MgSO_4$ and the solvents used were freshly distilled and purified before use. Petroleum ether, b.p. 60–80°, was used.

Extraction and isolation procedure : Shade-dried flowers (1.2 kg) of *E variegata* (collected from the main campus of the Indian Institute of Technology, Hauz Khas, New Delhi, in the month of April 1984) were extracted with petroleum ether (3 × 2 dm^3, 12 h). The defatted plant material was then extracted with acetone (3 × 2 dm^3, 12 h). The combined acetone extracts was concentrated under reduced pressure to give a greyish semisolid mass (13.4 g) which on maceration with methanol gave a solid. It showed six spots on TLC plates, but when subjected to column chromatography over silica gel, it afforded only two compounds A_1 and A_2. On the other hand, methanol-soluble part on concentration under reduced pressure and chromatography over neutral alumina yielded three major compounds A_5, A_3 and A_4.

Compound A_1 (78 mg) was eluted from a mixture of



Scheme 3 Suggested biogenetic pathways for the formation of erythrosotidienone (**4**)

petroleum ether-benzene (1 : 3) and crystallized from methanol as white silky needles, $[\alpha]_{25}$ –52, m.p. 168°. It was identified as stigmasterol (lit.[17,18], m.p. 170°), which was confirmed by preparation of its acetate (Py/Ac_2O); R.T., 48 h), a white powder, $[\alpha]_{25}$ –54, m.p. 143° (lit.[17,18], 143–144°).

Compound A_2 (42 mg) was eluted and crystallized from a mixture of benzene-chloroform (4 : 1) as white needles, m.p. 116°. It was established as cycloartenol (lit.[19,20], m.p. 115°) and was confirmed by preparation of its acetate derivative as white plates, m.p. 122° (lit.[19,20], 122°) and direct comparison with literature data.

Compound A_3 (**4**; 92 mg) was isolated as a light brown semisolid from benzene-chloroform (10 : 3) eluate and was found to be soluble in dilute HCl and H_2SO_4. It did not respond to the Liebermann-Burchard test and $FeCl_3$ reactions, but gave a clear pale yellow bright colour with Meyers reagent, and analyzed for $C_{17}H_{15}NO_3$ (Found : C, 72.39; H, 5.28; N, 4.90. Requires : C, 72.59; H, 5.34; N, 4.98%); ν_{max} 2930s, 2860s, 1740s, 1610s, 1460s, 1440s, 1380s, 1280–60bs, 1160s, 1130s, 1075s, 1040s, 975s, 960s, 860–40bs, 730s,sh, 720s, 675s, 645s, 610s, 580s cm^{-1}, ^1H nmr ($CDCl_3$) δ 3.63 (2H, t, *J* 1.5 and 4.5 Hz, C-10-H), 5.72 (1H, m, C-2-H), 5.94 (2H, s, O-CH_2-O), 7.32 (1H, s, C-14-H), 7.23 (1H, s, C-17-H), 6.62 (1H, s, C-7-H), 6.02 (1H, d, *J* 10.0 Hz, C-1-H), 1.87–3.27 (6H, m, methylene and methine protons); *m/z* (% rel. abundance) 281 (5.0), 280 (22.5), 279 (100), 267 (7.5), 265 (7.5), 253 (17.5), 227 (2.5), 226, 199 (15.4), 174 (15.0), 167 (10.0), 133 (5.0), 81 (31.6), 120 (8.0), 77 (5.0), 76 (80.0), 56 (27.3), 55 (42.5), ^{13}C nmr ($CDCl_3$) δ 184.0 (C=O), 152.0 (C-16), 152.4 (C-15), 132 (C-2), 131.0 (C-1), 129.6 (C-7), 128.3 (C-12), 128.1 (C-13), 114.0 (C-17), 110.1 (C-14), 104.0 (C-6), 102.8 (-O-CH_2-O), 68.1 (C-5), 40.3 (C-10), 32.0 (C-3), 30.4 (C-4), 23.2 (C-11).

Compound A_4 (**10**; 56 mg) was isolated from a mixture of benzene-chloroform (1 : 1) eluate, purified by preparative TLC using the same solvent system and crystallized from methanol as white needles, m.p.172°. It responded to the 2,4-DNP test for ketones and the Meyers test for alkaloids, but gave no reaction with $FeCl_3$. It analyzed for $C_{18}H_{19}NO_3$ (Found : C, 72.98; H, 6.50; N, 4.63. Requires : C, 72.72; H, 6.39; N, 4.71%); ν_{max} 2920s, 2880s, 1760s, 1600s, 1450s, 1380s, 1280–60bs, 1335s, 1280–60bs, 1250s, 1170s, 1110s,sh, 1075a, 1055s, 1025wb, 975s, 960s, 925s, 885w,sh, 835s, 800s, 765w,sh, 730s, 700s cm^{-1}; ^1H nmr ($CDCl_3$) δ 3.86 (3H, s, OCH_3), 3.36 (3H, s, OCH_3), 7.21 (1H, d, *J* 2.7 Hz, C-14-H), 7.32 (1H, dd, *J* 2.5 and 9.0 Hz, C-16 or C-15-H), 7.13 (1H, d, *J* 2.3 Hz, C-17-H), 6.62 (1H, s, C-7-H), 6.34 (1H, d, *J* 9.6 Hz, C-1-H), 6.02 (1H, dd, *J* 2.5 and 7.5 Hz, C-2-H), 1.92–3.76 (7H, m, methylene and methine protons); *m/z*(% rel. abundance) 297 (5), 296 (8), 295 (11), 269 (19), 255 (30), 239 (8), 235 (10), 213 (16), 185 (16), 83 ($CHCl_2^+$), 81 (43).

Compound A_5 (**13**; 52 mg) eluted from petroleum ether-benzene (5 : 4) eluates as a light yellow semisolid which colud not be crystallized. It responded positively to a 2,4-DNP test and the Meyers test ($HgCl_2$ and KI in H_2O), but gave no colour change with $FeCl_3$. It analyzed for $C_{19}H_{21}NO_4$ (Found : C, 69.56; H, 6.30; N; 4.18. Requires : C, 69.72; H, 6.42; N, 4.28%); ν_{max} 2930s, 2860s, 1760s, 1600s, 1475s, 1380s, 1310s, 1170s, 1030s, 980–70bs, 915s,sh, 885–60bs, 810s,sh, 740s, 720s, 695s, 610s, cm^{-1}; ^1H nmr ($CDCl_3$) δ 3.86, 3.62 and 3.36 (3H, s, each, 3 × OCH_3), 7.16 (1H, s, C-17-H), 6.97 (1H, s, C-14-H), 6.52 (1H, s, C-7-H), 6.34 (1H, d, *J* 5.5, C-1-H), 6.01 (dd, *J* 3.5 and 9.5 Hz, C-2-H), 3.18–1.72 (7H, m, methylene and methine protons); *m/z* (% rel. abundance) 327 (19), 299 (16), 269 (15), 268 (13), 265 (19), 264 (15), 243 (19), 159 (18), 82 (CCl_2), 81 (31).

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