The Biosynthesis of the *Erythrina* Alkaloids

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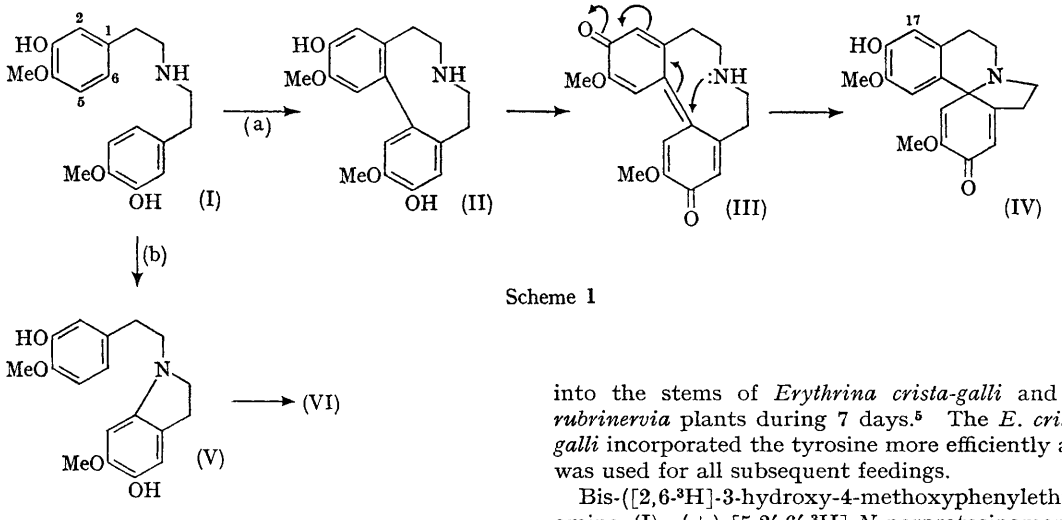
THE formation of erysodienone (IV) by the potassium ferricyanide oxidation[1,2] of the phenolic amine (I) provides *in vitro* support for the suggested biosynthesis of the *Erythrina* alkaloids *via* phenolic coupling of (I)[3] (Scheme 1). We now provide *in vivo* evidence which casts doubt upon this pathway and leads us to consider an alternative route (Scheme 2) originating with the benzyltetrahydroisoquinoline, *N*-norprotosinomenine (VI).[4] Chemical evidence for the later stages from the biphenyl derivative (II) is also presented, together with *in vivo* evidence for the subsequent transformations leading to erythraline (VIII).

[2-^{14}C]Tyrosine was fed through a cotton wick into the stems of *Erythrina crista-galli* and *E. rubrinervia* plants during 7 days.[5] The *E. crista-galli* incorporated the tyrosine more efficiently and was used for all subsequent feedings.



Scheme 1

Bis-([2,6-^{3}H]-3-hydroxy-4-methoxyphenylethyl)-amine (I), (±)-[5,2′,6′-^{3}H]-*N*-norprotosinomenine (VI), (±)-[17-^{3}H]erysodienone (IV), [2-^{3}H]erythratine (VIIa), and [2-^{3}H] epierythratine (VIIb) have been fed to *E. crista-galli* plants. The incorporations observed are listed in the Table.

Initial feeding experiments with the phenolic amine (I) gave low incorporations (0·0012% into

TABLE

Incorporation into erythraline and erythratine

Precursor	Activity (mc. fed).	Erythraline (VIII) (%)	Erythratine (VIIa) (%)
[2-^{14}C]Tyrosine.[a]	0·01	0·121	—
Bis-([2,6-^{3}H]-3-hydroxy-4-methoxyphenylethyl)amine[a] (I)	$6·5 \times 10^{-2}$	0·0043	0·0022
	$7·22 \times 10^{-2}$	0·0012	0·0055
(±)-[5,2′,6′-^{3}H]-*N*-Norprotosinomenine.[b] (VI)	$3·6 \times 10^{-2}$	0·24	0·020
	$5·63 \times 10^{-2}$	0·048	0·006
(±)-[17^{3}H]Erysodienone[b] (IV)	$2·35 \times 10^{-2}$	0·085	0·15
[2-^{3}H]Erythratine (VIIa)	$6·03 \times 10^{-3}$	0·041	—
	0·120	0·050	—
[2-^{3}H]Epierythratine (VIIb)	$5·3 \times 10^{-3}$	0·41	—
	0·20	0·28	—

[a] Incorporation into erythraline with an *E. rubrinervia* plant 0·031%.

[b] Labelled by base(self)-catalysed exchange in dimethylformamide–tritiated water at 100—120°, and the labelled position determined by n.m.r. studies on similarly deuterated materials. We thank Mrs. A. J. Kirby for generously providing *OO*-dibenzyl-*N*-norprotosinomenine.

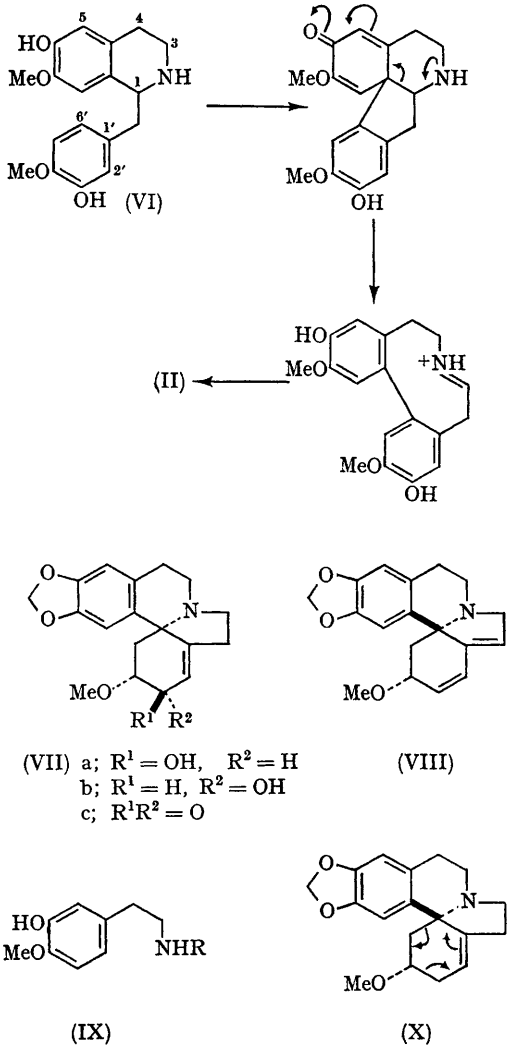
erythratine) which prompted a study of the alternative precursor (VI). Parallel feedings with (I) and (VI) in plants of the same age (4 months) showed clearly that (VI) was the precursor of erythraline and erythratine, rather than (I).

The suggested sequence from (VI) through Scheme 2 involves the biphenyl derivative (II). In order to synthesise (II) to test the feasibility of its cyclisation in the required fashion, the dienone (IV) was reduced with chromous chloride in acidic solution.[6] The product crystallised from chloroform as a chloroform solvate (identified by analysis) m.p. 149—150°, ν_{max}(CHCl$_3$) 3550, 3300, 1595 cm.$^{-1}$, λ_{max} (EtOH) 284 mμ (ε 7600), mass spectrum: *m/e* 315 (molecular ion), 300 (−CH$_3$), 284 (−OCH$_3$), 272 (loss of nitrogen bridge), 241 (base peak), n.m.r. spectrum τ 3·28 (2 protons, singlet), 3·36 (2 protons, singlet), 6·16 (6 protons, singlet), 6·6—7·6 (complex). Acetic anhydride–pyridine converted (II) into a triacetyl derivative, m.p. 215—217°, ν_{max}(CHCl$_3$) 1755, 1630 cm.$^{-1}$, mass spectrum *m/e* 441 (molecular ion), 399 (−CH$_2$CO), 357 (−2 × CH$_2$CO), 271 (base peak) and the appropriate n.m.r. spectrum.

Mr. R. B. Boar has shown that oxidation of the biphenyl (II) with potassium ferricyanide under the conditions used for the cyclisation of (I)[2] gave an 80% yield of the dienone (IV), identified by m.p., t.l.c., and spectroscopic data. This ready chemical cyclisation lends support to a biosynthetic sequence involving (II). Also the chemical cyclisation of (I) may involve (II) (Scheme 1a) rather than the dihydroindole derivative (V)[1] (Scheme 1b), since oxidation of the model phenolic amines (IX; R=H or CH$_2$Ph) under the conditions used for cyclisation of (I) gave no dihydroindole derivatives, although cyclisations have been effected under similar conditions, with 2,5-dihydroxyphenylalanine, in which *p*-quinone formation is possible.[7]

Both Scheme 1 and Scheme 2 involve the dienone

Scheme 2



(IV), as the first cyclisation product with the erythrinan skeleton, and in keeping with this, it was found to be incorporated comparatively efficiently into erythratine (VIIa) although less so into erythraline (VIII).

Erythratine (VIIa) has been converted chemically into erythraline,[8] but the *in vivo* transformation has not yet been proved. Tritiated erythratine (VIIa) and epierythratine (VIIb) were prepared by the sodium borotritiide reduction of erythratinone (VIIc). As indicated in the Table, epierythratine (VIIb) was more efficiently incorporated into erythraline (VIII) than erythratine (VIIa) but the latter still had a finite incorporation. This may be due to equilibration of the alkaloids in the plant. It was found that acid treatment at 100° of erythratine (VIIa) gave an equilibrium mixture of 95% erythratine and 5% epierythratine.

These preliminary results indicate a novel biosynthetic route to the erythrinan ring system, but final proof awaits the more definitive multiple labelling and degradative experiments which are currently in progress.

In our final clarification[8] of the constitutions of the *Erythrina* alkaloids we did not make reference to erythramine,[9] in which the position of the ethylenic linkage has not been defined. An analysis of the n.m.r. spectrum of erythramine indicated the constitution (X). This was confirmed by mass-spectrometric considerations. The mass spectra of *Erythrina* alkaloids containing a 1(6)-ethylenic linkage (erythratine, erythratine benzoate, epierythratine, erythratinone, dihydroerysodine[2]) all undergo a reverse Diels–Alder reaction [(X), see arrows] on electron impact giving rise to strong $M-58$ and $M-59$ peaks. The other *Erythrina* alkaloids do not exhibit this fragmentation. Erythramine (M^+ 299) shows a strong $M-58$ peak consistent with the above formulation.

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[4] Correct analytical data or accurate mass measurements have been made on all new compounds.
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