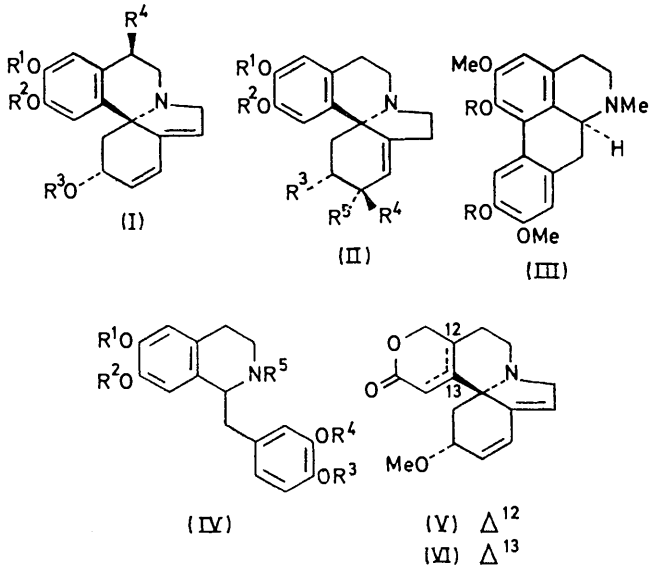
Phenol Oxidation and Biosynthesis. Part XXII.[1] The Alkaloids of *Erythrina lysistemon, E. abyssinica, E. poeppigiana, E. fusca,* and *E. lithosperma;* the Structure of Erythratidine

By **Derek H. R. Barton, Abeysinghe A. L. Gunatilaka, Roy M. Letcher, Awa M. F. T. Lobo,** and **David A. Widdowson,*** Chemistry Department, Imperial College, London SW7 2AY

Erythrina lysistemon, E. abyssinica, E. poeppigiana, E. fusca, and *E. lithosperma* have been examined for alkaloids of biogenetic interest. In addition to the known Erythrina alkaloids, α- and β-erythroidine, eryosdine, erythraline, erythratine, and erysotrine, new alkaloids erythristemine, erythratidinone, and 3-demethoxyerythratidinone were isolated and characterised. The aporphine alkaloid isoboldine, and the benzyltetrahydroisoquinoline alkaloids (−)-orientaline and (+)-*N*-nororientaline were also isolated. A partial synthesis of erythratidine indicated a 2*S* configuration. A plausible biogenetic scheme for the new alkaloids is discussed.

INVESTIGATIONS on the biosynthesis of Erythrina alkaloids[1] have shown the intermediacy of (+)-*N*-norprotosinomenine, 5,6,8,9-tetrahydro-2,12-dimethoxy-7*H*-dibenz[*d*,*f*]azonine-3,11-diol and of erysodienone. Recently, erysodienone and (+)-*N*-norprotosinomenine have been reported as constituents of *E. lithosperma*.[2] The azonine remains unknown in nature, although the *N*-methyl analogue has been isolated from *E. xbidwilli*.[2] In an attempt to detect these or other intermediates of biogenetic interest, we have investigated the alkaloidal constituents of a number of Erythrina species some of which have been examined previously.

Erythrina lysistemon.[3]—This was previously unexamined (but see ref. 4). The bases were extracted with 0·04N-hydrochloric acid and separated by column chromatography on alumina (Grade III). In addition to erysodine (I; $R^1 = R^4 = H$, $R^2 = R^3 = Me$), m.p. 202—204°, in 0·004% yield (identified by comparison with authentic alkaloid) a new compound, designated erythristemine, was isolated in 0·0024% yield. Erythristemine crystallised as pale yellow prisms m.p. 127—129° (from light petroleum), $[\alpha]_D^{22}$ +189°. It gave a picrate, m.p. 145—150°, and a 2-bromo-4,6-dinitrophenolate, m.p. 144—146°. This latter was used for an *X*-ray determination of the structure of erythristemine.[3]



Microanalysis of the derivatives indicated a molecular formula of $C_{20}H_{25}NO_4$ for the parent base and this was

[1] Part XXI, D. H. R. Barton, R. B. Boar, and D. A. Widdowson, *J. Chem. Soc.* (*C*), 1970, 1213, and references cited therein.

[2] S. Ghosal, S. K. Majumdar, and A. Chakraborti, *Austral. J. Chem.*, 1971, **24**, 2733; K. Ito, H. Furukawa, and H. Tanaka, *Chem. Comm.*, 1970, 1076; *Chem. and Pharm. Bull.* (*Japan*), 1971, **19**, 1509.

[3] Preliminary report, D. H. R. Barton, P. N. Jenkins, R. Letcher, D. A. Widdowson, E. Hough, and D. Rogers, *Chem. Comm.*, 1970, 391.

[4] R. Letcher, *J. Chem. Soc.* (*C*), 1971, 652.

confirmed by an accurate mass measurement of the molecular ion in the mass spectrum. The i.r. spectrum ($\nu_{max.}$ 1610 cm^{-1}) and the u.v. spectrum [$\lambda_{max.}$ (EtOH) 283 (ε 3100) and 235 nm (20,000)] were almost identical with those of erythraline.[5] The mass spectrum showed significant ions at *m/e* 343 (M^+), 328, 312, 311 (100%), 310, 296, and 280. The fragmentation pattern was typically that of a 1,6-diene type Erythrina alkaloid [6] with an additional oxygen function.

The n.m.r. spectrum showed signals for four methoxy-groups, two singlet aromatic protons, and three olefinic proton multiplets. On the assumption of an erythrinan skeleton, INDOR experiments,[7] which have been discussed in full elsewhere,[3,*] allowed the assignment of all the remaining aliphatic protons to the 11-methoxyerysotrine structure (I; $R^1 = R^2 = R^3 = Me$; R^4 = OMe) for erythristemine. The remaining ambiguity, the configuration at C-11 was resolved by the *X*-ray determination.[3]

Erythrina abyssinica.—The seeds of this plant had been examined [8] and erysopine (I; $R^1 = R^2 = R^4 = H$, $R^3 = Me$), erysodine (I; $R^1 = R^4 = H$, $R^2 = R^3 = Me$), erythraline (I; $R^1,R^2 = CH_2$, $R^3 = Me$, $R^4 = H$), and glucoerysodine (I; R^1 = 1-β-glucosyl, $R^2 = R^3 = Me$, $R^4 = H$) detected. Extraction of the macerated leaves with 0·02N-hydrochloric acid gave a crude basic fraction which was separated by column chromatography over alumina (Grade III) into five alkaloidal components. First, elution with 10% ethyl acetate–benzene gave erythraline (I; $R^1,R^2 = CH_2$, $R^3 = Me$, $R^4 = H$) in 0·00023% yield which was characterised as the hydrobromide, m.p. 241—245°, $[\alpha]_D$ +219°, identical with authentic material.

Further elution with this solvent system gave erythristemine in 0·00013% yield, m.p. 126—128° $[\alpha]_D$ +183°, identical with the material obtained from *E. lysistemon*.

Ethyl acetate eluted erythratine (II; $R^1,R^2 = CH_2$, R^3 = OMe, R^4 = OH, $R^5 = H$), m.p. 176—178°, in 0·00025% yield, identical with authentic alkaloid.

Finally elution with ethanol–ethyl acetate gave a mixture of two bases, chromatographically different to any available Erythrina alkaloid. These were separated by preparative t.l.c.

The faster running (R_F 0·5) component, m.p. 118—120°, analysed for $C_{19}H_{21}NO_4$ by mass spectrometry and *via* the crystalline picrate, m.p. 197—198°. It had a u.v. spectrum characteristic of an aporphine alkaloid [9] and only two aromatic protons in the n.m.r. spectrum, which is consistent with a tetraoxygenated aporphine system. Methylation of the base with diazomethane in ether–methanol gave (+)-glaucine (III; R = Me).[10] Finally the isolated alkaloid was identified as isoboldine (III; R = H) by spectroscopic and chromatographic comparison with synthetic racemic material. This is the first instance of an aporphine alkaloid in an Erythrina species.

The slower running component (R_F 0·3) was purified as the amorphous methiodide. The u.v. spectrum ($\lambda_{max.}$ 283 nm), was consistent with the presence of dioxygenated aromatic rings [11] and a reversible base-induced red shift of 30 nm indicated the presence of free phenolic groups. The high resolution mass spectrum indicated a molecular formula of $C_{20}H_{25}NO_4$ for the thermal Hofmann elimination product and a base peak at *m/e* 192 was indicative of a benzyltetrahydroisoquinoline alkaloid with one methoxy- and one hydroxy-group on each aromatic ring.[12] The n.m.r. spectrum was assignable to a 3′,4′,6,7-tetraoxygenated system [as (IV)] [13] (see Experimental section) with two methoxy and one *N*-methyl groups. A small sample of free base was purified by column chromatography on alumina and crystallisation of the perchlorate salt. This had a m.p. identical to that reported for orientaline perchlorate,[14] an alkaloid recently detected on *E. arborescens*.[15] (±)-Orientaline (IV; $R^1 = R^4 = R^5 =$ Me, $R^2 = R^3 = H$) was synthesised from the available (±)-*N*-norientaline and found to be chromatographically identical with the natural material. This was chromatographically distinct from the other three isomers with one methoxy- and one hydroxy-group in each aromatic ring with the oxygenation pattern as in (IV), *i.e.* reticuline (IV; $R^1 = R^3 = R^5 = Me$, $R^2 = R^4 = H$), protosinomenine (IV; $R^1 = R^4 = H$, $R^2 = R^3 = R^5 = Me$) and the wholly synthetic compound (IV; $R^1 = R^3 = H$, $R^2 = R^4 = R^5 = Me$).

Finally the oxygenation pattern was confirmed and the absolute stereochemistry assigned by *O*-methylation of the natural base with diazomethane to give partially racemic (−)-laudanosine,[16] and *N*-methylation to the methiodide, m.p. 219—220°. This established the orientaline to be of the 1*R* configuration. The origin of the partial racemisation is not evident here, but the biological interconversion of benzyltetrahydroisoquinoline enantiomers *via* the imine is well authenticated in other species.[17]

* The coupling constant referred to as $J_{4a,7}$ in ref. 3 should read $J_{4e,7}$.

[5] V. Prelog, K. Wiesner, H. G. Khorana, and G. W. Kenner, *Helv. Chim. Acta*, 1949, **32**, 453.
[6] R. B. Boar and D. A. Widdowson, *J. Chem. Soc.* (*B*), 1970, 1591.
[7] V. J. de Kowalewski, D. G. de Kowalewski, and E. C. Ferra, *J. Mol. Spectroscopy*, 1966, **20**, 203.
[8] K. Folkers and F. Koniuszy, *J. Amer. Chem. Soc.*, 1940, **62**, 1677.
[9] A. W. Sangster and K. L. Stuart, *Chem. Revs.*, 1965, **65**, 69.
[10] R. H. F. Manske, *Canad. J. Res.*, 1938, **8**, 592.
[11] L. Doub and J. M. Vanderbelt, *J. Amer. Chem. Soc.*, 1949, **71**, 2414.
[12] H. Budzikiewicz, C. Djerassi, and D. H. Williams, 'Structure Elucidation of Natural Products by Mass Spectrometry,' Holden-Day, San Francisco, 1964, vol. 1, p. 173.
[13] D. H. R. Barton, R. H. Hesse, and G. W. Kirby, *J. Chem. Soc.*, 1965, 6379.
[14] A. R. Battersby, T. H. Brown, and J. H. Clements, *J. Chem. Soc.*, 1965, 4550.
[15] S. Ghosal, A. Chakraborti, and R. S. Srivastava, *Phytochemistry*, 1972, **11**, 2101.
[16] M. Tomita and J. Kunitomo, *Yakugaku Zasshi*, 1962, **82**, 734.
[17] A. R. Battersby, D. M. Foulkes, and R. Binks, *J. Chem. Soc.*, 1965, 3323.

E. poeppigiana *Walp.*—This species had been examined previously [8] and erysodine, erysovine, and erysothiovine isolated from the seeds. Acid extraction of the dried leaves gave a mixture of α- (V) and β-erythroidine (VI) from the weakly basic fraction and a new alkaloid from the strongly basic fraction. This was purified by column chromatography on alumina and by crystallisation of the hydrobromide salt, m.p. 257°.

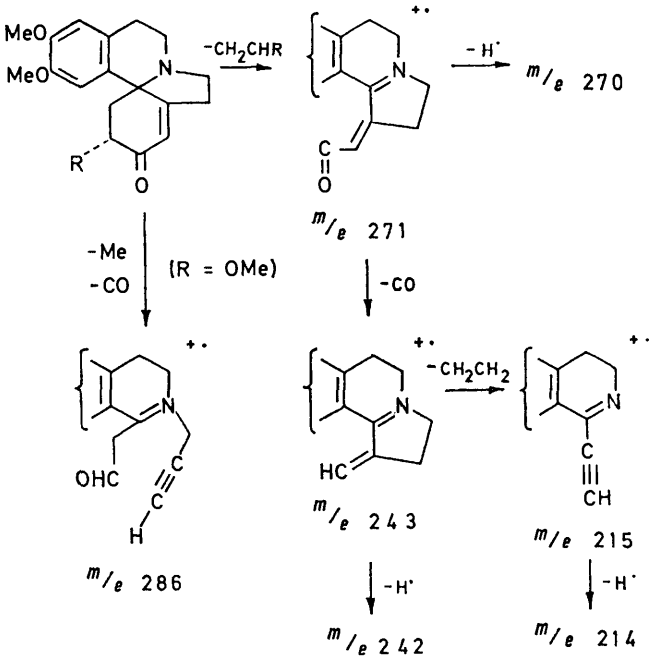
Microanalysis gave a molecular formula of $C_{18}H_{22}BrNO_4$. The free base, m.p. 145—147°, $[\alpha]_D$ +42°, exchanged two aromatic protons on being heated with deuterium oxide.[18] The compound was identified as (+)-*N*-nororientaline (IV; $R^1 = R^4 = Me$, $R^2 = R^3 = R^5 = H$) by spectroscopic and chromatographic comparison with authentic (±)-*N*-nororientaline and its hydrochloride. In addition *N*-methylation gave orientaline, isolated as the perchlorate, m.p. 128—130° and characterised as orientaline methiodide, m.p. 125—127°, $[\alpha]_D$ −57°. The stereochemistry of the isolated material was thus established as 1*R*. *N*-Nororientaline has been reported recently in other Erythrina species.[2]

E. fusca *Lour.*—The seeds of this have been reported [19,20] to contain erythraline, erysopine, erysovine, and erysodine. The leaves were acid-extracted and the basic material was isolated. Only one alkaloid was detectable by t.l.c. This was purified *via* the picrate and identified as erysotrine [21] (I; $R^1 = R^2 = R^3 =$ Me, $R^4 = H$) by comparison with authentic material prepared by *O*-methylation of erysovine with diazomethane. Erysotrine, previously only known as a synthetic base, has recently been isolated from *E. variegata*.[21].

E. lithosperma *Blume.*—The seeds of this species reportedly [2] contain among others, the biosynthetically important alkaloids *N*-norprotosinomenine and erysodienone. Dried leaf material of a smooth variety of the plant * was acid extracted as before and the crude basic material was chromatographed over alumina. Elution with benzene–ethyl acetate (2 : 1) gave traces of erythraline (I; $R^1,R^2 = CH_2$, $R^3 = Me$, $R^4 = H$) together with erysotrine (I; $R^1 = R^2 = R^3 = Me$, $R^4 = H$) [21] in 0·22% yield which was characterised as the picrate, m.p. 159—161°, $[\alpha]_D$ +142°. In addition two new ketonic alkaloids were isolated which, on the following evidence, were shown to be erythratidinone (II; $R^1 = R^2 = Me$, $R^3 = OMe$, $R^4,R^5 = O$) in 0·12% yield, and 3-demethoxyerythratidinone (II; $R^1 = R^2 = Me$, $R^3 = H$, $R^4,R^5 = O$) in 0·016% yield.

Erythratidinone (II; $R^1 = R^2 = Me$, $R^3 = MeO$, $R^4,R^5 = O$), $C_{19}H_{23}NO_4$ by microanalysis, had m.p. 119—120°, $[\alpha]_D$ +358°. It showed enone absorption in the u.v. spectrum ($\lambda_{max.}$ 231 nm) in addition to the usual dioxyaryl absorption ($\lambda_{max.}$ 284 nm).[11] The n.m.r. spectrum indicated the presence of three methoxy-groups, two aromatic protons [τ 3·40 (s) and 3·62 (s)], the α-proton of an α,β-unsaturated ketone (τ 4·08), and a proton α to oxygen (τ 5·98), in addition to the methylene envelope.

INDOR decoupling experiments [7] enabled the n.m.r. absorptions to be assigned further. Irradiation at τ 7·92 sharpened the olefinic proton (1-H) at τ 4·08 by removing the coupling between 1- and 7-H. The quartet at τ 5·98 (3-H) collapsed to a doublet (J 5·5 Hz) on irradiation at τ 7·69. By monitoring each line of the 3-H quartet using the INDOR technique, seven responses in the τ 7·44—7·68 region were detected. The quartet centred around τ 7·44 had J_1 12·0 and J_2 5·5 Hz, and the three line absorption centred on τ 7·68 had $J_1 = J_2 = 12·0$ Hz. These couplings are consistent with an axial proton at C-3 coupled to the 4e-H (τ 7·44) and 4a-H (τ 7·68) by 5·5 and 12·0 Hz respectively, and in addition a geminal 4-H coupling of 12·0 Hz. The 3-methoxy-group is therefore in the equatorial conformation, as with the other Erythrina alkaloids.[22] The mass spectrum of erythratidinone showed the characteristic fragmentation pattern of the 1(6)-en-2-one alkaloids [6] (Scheme 1) and is almost identical to that of erythratinone (II; $R^1,R^2 = CH_2$, $R^3 = OMe$, $R^4,R^5 = O$) when allowance is made for the mass difference of 16. The picrate of erythratidinone, m.p.



SCHEME 1 Mass spectral fragmentations of enone-alkaloids

205—207°, showed unsaturated ketone absorption in the i.r. spectrum ($\nu_{max.}$ 1698 cm^{-1}) confirming the assignment

* A thorned variety was also available. It contained only erysotrine.

[18] G. W. Kirby and L. Ogunkoya, *J. Chem. Soc.*, 1965, 6914.
[19] K. Folkers, J. Shavel, and F. Koniuszy, *J. Amer. Chem. Soc.*, 1941, **63**, 1544.
[20] K. Folkers and F. Koniuszy, *J. Amer. Chem. Soc.*, 1940, **62**, 436.
[21] S. Ghosal, D. K. Ghosh, and S. K. Dutta, *Phytochemistry*, 1970, **9**, 2397.
[22] V. Boekelheide and G. R. Wenzinger, *J. Org. Chem.*, 1964, **29**, 1307.

from the u.v. spectrum. These data are consistent with structure (II; $R^1 = R^2 = Me$, $R^3 = OMe$, $R^4,R^5 = O$) for this alkaloid.

Erythratidinone was reduced with sodium borohydride to give a C-2 epimeric mixture of alcohols. One of these, m.p. 120—120·5°, $[\alpha]_D$ +273° (EtOH), +258° ($CHCl_3$), had properties identical with those reported for erythratidine, an alkaloid of partially defined structure isolated from *E. falcata*.[23] The C-2 epimer, m.p. 67—68° (solvate), had $[\alpha]_D$ +142°. As erythratidine has a more positive rotation than its epimer, it must, by Mills' rule [24] have the 2*S* absolute configuration, as in (II; $R^4 = H$, $R^5 = OH$).

The n.m.r. spectrum of erythratidine shows the olefinic proton absorption of 1-H at τ 4·19, with $J_{1,2}$ 4·25 Hz, and the 2-H absorption at τ 5·57 with $J_{2,3}$ 4·25 Hz. Since 3-H has been shown to be axial these data are consistent only with a pseudoequatorial proton at C-2, and are comparable with the 3—4 Hz coupling observed [22] in epierythratine (II; $R^1,R^2 = CH_2$, $R^3 = OMe$, $R^4 = H$, $R^5 = OH$) and not the 7·5 Hz 2-H–3-H coupling of erythratine (II; $R^1,R^2 = CH_2$, $R^3 = OMe$, $R^4 = OH$, $R^5 = H$). It follows that erythratidine and hence erythratidinone have the absolute configuration at C-3 as represented in (II).

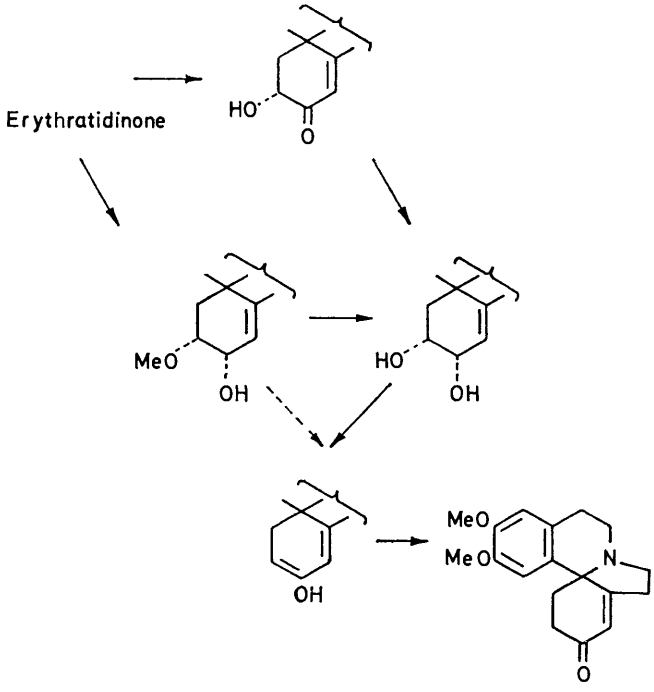
Finally, the configuration at C-5 was shown to be as (II) and that at C-3 confirmed, by dehydration of erythratidine, albeit in low yield, to erysotrine, an alkaloid of known absolute stereochemistry.[21] The dehydration was effected with methanesulphonyl chloride in pyridine. The major product was the 2-chloro-derivative (II; $R^1 = R^2 = Me$, $R^3 = OMe$, $R^4,R^5 = H,Cl$).

The second new alkaloid was assigned the 3-demethoxyerythratidinone structure (II; $R^1 = R^2 = Me$, $R^3 = H$, $R^4,R^5 = O$) on the basis of spectral studies. The base was obtained after p.l.c. purification as yellow prisms, m.p. 111—112°, $[\alpha]_D$ +325°, and as a picrate, m.p. 200—202°, in 0·016% yield based on dry weight of leaves. Microanalysis gave a formula of $C_{18}H_{21}NO_3$ for the free base, which showed enone absorption in the i.r. spectrum at 1667 cm^{-1}. The n.m.r. spectrum had absorptions due to two aromatic protons, the α-proton of an α,β-unsaturated ketone and two methoxy-groups in addition to the methylene envelope. The mass spectrum of the alkaloid showed a fragmentation pattern almost identical with erythratidinone below *m/e* 271. The only difference lay in the loss of the initial fragment of *m/e* 28 in the retro-Diels–Alder process, rather than a fragment of *m/e* 58 (see Scheme 1).

The biogenetic origin of erythratidine can be considered to be analogous to that of epierythratine.[22] The greater efficiency of epierythratine over erythratine as a precursor of erythraline [22] suggested that erythratine was a 'shunt' product. The configurational correlation of erythratidine with epierythratine adds further weight to this proposal and implies that erythratidine is the precursor of erysotrine.

[23] V. Deulofeu, *Chem. Ber.*, 1952, **85**, 620.
[24] J. A. Mills, *J. Chem. Soc.*, 1952, 4976.

3-Demethoxyerythratidinone is unique in this species (*E. lithosperma*) in its lack of the 3-methoxy-group. This implies a late stage modification of the normal biosynthetic pathway. Some plausible routes are indicated in Scheme 2.



SCHEME 2 Biogenesis of 3-demethoxyerythratidinone

The unique occurrence of the C-11 substituted erythristemine in *E. lysistemon* again suggests a late stage benzylic hydroxylation. Other C-11 oxygenated alkaloids are now known [25] and in all cases, the alkaloids are of the 'biogenetically complete' 1,6-diene types.

EXPERIMENTAL

Unless otherwise stated, i.r. spectra were recorded for chloroform, u.v. spectra for ethanol, and n.m.r. spectra for deuteriochloroform solutions. T.l.c. was carried out on alumina GF plates. For preparative work these were 1 mm thick.

Extraction of E. lysistemon.—Dry leaves (5 kg) of *E. lysistemon* were stirred intermittently with 0·04N-hydrochloric acid (20 l) for 2 days. This procedure was twice repeated, the combined extracts (60 l) were neutralised (Na_2CO_3), and washed with methylene chloride (3 × 10 l). The washings were dried (Na_2SO_4), concentrated, and the residual oil was acidified with 0·5N-hydrochloric acid (400 ml). The aqueous solution was washed with light petroleum, basified ($NaHCO_3$), and extracted with chloroform (3 × 200 ml). Evaporation of the combined dried (Na_2SO_4) extracts gave a gum which was chromatographed on an alumina (Grade III) column. Elution with benzene–ethyl acetate (9 : 1) gave an alkaloidal component (erythristemine) (300 mg) with t.l.c. properties different from any reported Erythrina alkaloid. Further elution with ethyl

[25] K. Ito, H. Farukawa, and H. Tanaka, *Chem. Comm.*, 1970, 1076; R. M. Letcher, *J. Chem. Soc.* (*C*), 1971, 652.

acetate–ethanol (9 : 1) gave, as the only other detectable alkaloid, erysodine (I; $R^1 = R^4 = H$, $R^2 = R^3 = Me$) (200 mg), m.p. and mixed m.p. 202—204° (from EtOH), with spectral and t.l.c. properties identical with those of an authentic specimen.

Erythristemine.—The crude unidentified alkaloid isolated above was rechromatographed over alumina (Grade III). Elution with benzene–ethyl acetate (9 : 1) gave pale yellow prisms of *erythristemine* (I, $R^1 = R^2 = R^3 = Me$, $R^4 =$ OMe) (120 mg), m.p. 127—129° (from light petroleum), $[\alpha]_D^{22}$ +189° (*c* 0·4 in $CHCl_3$), $\nu_{max.}$ 1610 cm^{-1}, $\lambda_{max.}$ 235 (log ε 4·3) and 283 nm (3·5), τ 2·91 (1H, s, 14-H), 3·07 (1H, s, 17-H), 3·68 (1H, dd, J_1 10, J_2 2 Hz, 2-H), 4·12 (1H, d, *J* 10 Hz, 1-H), 4·62br (1H, s, 7-H), 5·94 (1H, X of ABMX, J_{AX} 5·5, J_{BX} 10·5, J_{MX} 2 Hz, 3-H), 6·06 (1H, dd, J_1 4, J_2 4 Hz, 11-H), 6·03 (1H, A of ABX, J_{AB} 15, J_{AX} 2·5 Hz, 8a-H or 8e-H), 6·33 (1H, B of ABX, J_{BA} 15, J_{BX} 2·5 Hz, 8e-H or 8a-H), 6·57 (6H, s, OMe) 6·79 (3H, s, OMe), 6·98 (3H, s, OMe), 6·5—7·2 (2H, overlapping dd, J_1 14, J_2 4 Hz, 10-H), 7·51 (1H, dd, J_1 10·5, J_2 5·5 Hz, 4e-H), and 7·92 (1H, dd, J_1 10·5, J_2 10·5 Hz, 4a-H), *m/e* 343 (M^+), 328, 312, 311 (100%), 310, 296, and 280 (Found: C, 69·65; H, 7·3; N, 3·85%; M^+, 343·1789. $C_{20}H_{25}NO_4$ requires C, 69·95; H, 7·35; N, 4·1%; M^+, 343·1783).

Erythristemine (13 mg) and picric acid (8·7 mg) in ethanol (2 ml) gave *erythristemine picrate* (6 mg), m.p. 145—150° (from EtOH) (Found: C, 55·1; H, 5·1; N, 9·45. $C_{26}H_{28}O_{11}$ requires C, 54·55; H, 4·9; N, 9·8%).

Erythristemine (15 mg) and 2-bromo-4,6-dinitrophenol (11·5 mg) in ethanol (2 ml) gave *erythristemine 2-bromo-4,6-dinitrophenolate* (20 mg), m.p. 144—146° (from EtOH) (Found: C, 52·15; H, 4·75; N, 6·5. $C_{26}H_{28}BrN_3O_9$ requires C, 51·5; H, 4·65; N, 6·95%).

Extraction of E. abyssinica.—Powdered dried leaves of *E. abyssinica* (10·0 kg) were stirred for 3 days in 0·02N-hydrochloric acid (120 l). The acidic solution was filtered through Celite and washed with light petroleum (b.p. 40—60°; 6 × 4 l). The acidic extract was basified ($NaHCO_3$) to pH 9 and repeatedly extracted with chloroform (8 × 4·8 l). The combined extracts were dried (Na_2SO_4) and evaporated under reduced pressure, and the alkaloidal residue (11·25 g) chromatographed on alumina (Grade III). Elution with ethyl acetate–benzene (1 : 10) gave erythraline (I; $R^1,R^2 = CH_2$, $R^3 = Me$, $R^4 = H$), isolated as the hydrobromide (23 mg, 0·00023%), m.p. 241—245° (decomp.) (from EtOH), $[\alpha]_D$ +219° (*c* 0·1 in H_2O) [lit.,[22] m.p. 243° (decomp.), $[\alpha]_D$ +217° (H_2O)]. The free base had M^+ 297 and a fragmentation pattern identical with that of authentic material.[6]

Further elution of the column with the same solvent gave a crude alkaloid which was purified by preparative t.l.c. to yield erythristemine (I; $R^1 = R^2 = R^3 = Me$, $R^4 =$ OMe) (12·5 mg, 0·00013%), m.p. 126—128° (from light petroleum), mixed m.p. 124—127° with an authentic specimen (lit.,[3] m.p. 127—129°), $[\alpha]_D$ +183° (*c* 0·13 in $CHCl_3$).

Fractions eluted with ethyl acetate, after purification by preparative t.l.c., gave erythratine (II; $R^1,R^2 = CH_2$, $R^3 =$ OMe, $R^4 =$ OH, $R^5 = H$) (25·0 mg, 0·00025%), m.p. 176—178° (from EtOAc), mixed m.p. 173—175° with an authentic specimen (lit.,[20] m.p. 172—174°). The mass spectrum, with M^+ 315, was identical with that of authentic material.[6]

Elution with an increasing concentration of ethanol in ethyl acetate gave a mixture of two alkaloids which were separated by preparative t.l.c. [$CHCl_3$–MeOH (20 : 1)] and were identified as isoboldine (III; R = H), R_F 0·5, and orientaline (IV; R = H), R_F 0·3 (see below).

Isoboldine (III; R = H).—This was isolated as the hydrochloride (83 mg, 0·0008%), m.p. >300° (from MeOH–Et_2O) (lit.,[26] m.p. >300°). The free base had m.p. 118—120° (from $CHCl_3$), (lit.,[26] m.p. 118—120°). The u.v.[27] and n.m.r.[26] spectra were identical with those reported for isoboldine. The compound was also chromatographically indistinguishable from authentic racemic isoboldine.

Natural isoboldine (30 mg) in absolute methanol (5 ml) was methylated with excess of ethereal diazomethane during 16 h at room temperature. Purification of the product by t.l.c. gave (+)-glaucine (22 mg), m.p. 120—123° (from Et_2O), $[\alpha]_D$ +102° (*c* 0·7 in $CHCl_3$) [lit.,[10] m.p. 119—120°, $[\alpha]_D$ +120° ($CHCl_3$)]. The product had u.v.,[28] n.m.r.,[29] and mass [30] spectra identical with those reported for glaucine.

Orientaline (IV; $R^1 = R^4 = R^5 = Me$, $R^2 = R^3 = H$).—This was isolated as the *methiodide*, an amorphous solid (102 mg, 0·001%), $[\alpha]_D$ −35·5° (*c* 0·2 in Me_2CO), $\lambda_{max.}$ 283 (ε 5400) and 225infl nm (17,000), $\lambda_{max.}$ (NaOH–EtOH) 310 (ε 6,900) and 252 nm (14,800), τ([$^2H_6$]acetone) 3·17br (2H, s, 2- and 5-H), 3·39 (1H, d, *J* 8·0 Hz, 5′-H), 3·52 (1H, dd, J_1 8·0, J_2 2·0 Hz, 6′-H), 3·98 (1H, s, 8-H) 4·90 (1H, dd, J_1 9·0, J_2 4·0 Hz, 1-H), 6·22 (3H, s, OMe), 6·27 (3H, s, OMe), 6·93br (3H, s, NMe), 6·67br (3H, s, NMe), and 6·10—6·90 (6H, complex, aliphatics), *m/e* 343 (M^+), 312, 206, 192 (100%), 177, 149, and 137 [Found: M^+, 343·1775. $C_{20}H_{25}NO_4$ (Hofmann elimination product) requires *M*, 343·1783].

The free base was obtained as a gum from a sample of crude alkaloid by column chromatography on alumina. It had identical chromatographic properties with those of a sample of orientaline obtained by *N*-methylation of authentic *N*-nororientaline with methyl iodide. The base was purified as orientaline perchlorate, m.p. 123—126° (from MeOH–Et_2O) (lit.,[14] m.p. 127°).

Orientaline (40 mg) in absolute methanol (7·5 ml) was methylated with excess of ethereal diazomethane, during 16 h at room temperature. The product was purified by t.l.c. to yield partially racemic laudanosine (IV; R^{1-5} = Me) as a gum (33 mg), $[\alpha]_D$ −11·5° (*c* 0·2 in $CHCl_3$) (lit.[16] $[\alpha]_D$ −52° in $CHCl_3$).

The product was converted into the methiodide, m.p. 219—220° (from Me_2CO) (lit.,[31] m.p. 218—221°) (Found: C, 53·7; H, 6·3; N, 2·8. $C_{22}H_{30}INO_4$ requires C, 53·9; H, 6·5; N, 2·5%) (Found: M^+, 371·2098. $C_{22}H_{29}NO_4$ requires *M*, 371·2106).

Extraction of E. poeppigiana *Walp.*—Powdered leaves of *E. poeppigiana* Walp. (2·53 kg) were extracted as above with 0·05N-hydrochloric acid (18 l). After the acidic extract had been washed with light petroleum, it was

[26] P. S. Clezy, E. Gellert, D. Y. K. Lau, and A. W. Nichol, *Austral. J. Chem.*, 1966, **19**, 135.
[27] W. W. C. Chan and P. Maitland, *J. Chem. Soc.* (*C*), 1966, 753.
[28] M. Shamma, *Experientia*, 1960, **16**, 484.
[29] A. H. Jackson and J. A. Martin, *J. Chem. Soc.* (*C*), 1966, 2061.
[30] A. H. Jackson and J. A. Martin, *J. Chem. Soc.* (*C*), 1966, 2181.
[31] von H. Corrodi and E. Hardegger, *Helv. Chim. Acta*, 1956, **39**, 889.

further extracted with chloroform (6 × 700 ml). Evaporation of the dried (Na_2SO_4) solution gave a yellow gum (3·25 g, 0·13%). Chromatography over alumina (Grade III) gave a mixture of α- (V) and β-erythroidine (VI) (272 mg) which was further purified *via* the hydrobromide salts to m.p. 220—221° (decomp.) (from MeOH–Et_2O) [lit.,[3] m.p. 220—222° (decomp.)[32] (α-erythroidine), 222·5° (decomp.)[33] (β-erythroidine)]. This material was spectroscopically and chromatographically identical with a mixture of the hydrobromides of authentic alkaloids.

The acidic extract above was basified ($NaHCO_3$) to pH 8·2 and repeatedly extracted with chloroform (10 × 700 ml). The combined extracts were dried (Na_2SO_4) and evaporated to yield a crude alkaloidal fraction (2·83 g, 0·11%).

N-Nororientaline (IV; $R^1 = R^4 = Me$, $R^2 = R^3 = R^5 = H$).—Column chromatography of the crude alkaloidal fraction above on alumina (Grade III) gave on elution with benzene–ethyl acetate (1 : 1), α- and β-erythroidines (15 mg) isolated as a mixture of the hydrobromide salts. In addition, unresolved traces of an alkaloid with R_F identical to erythristemine could be detected by t.l.c.

Elution with ethyl acetate–ethanol (3 : 2) gave crude *N*-nororientaline (IV; $R^1 = R^4 = Me$, $R^2 = R^3 = R^5 = H$), isolated as the hydrobromide salt (433 mg, 0·017%), m.p. 257° (decomp.) (from MeOH–Et_2O), $\nu_{max.}$ (Nujol) 3300, 3200, 3100, 1605, and 1525 cm^{-1}, $\lambda_{max.}$ 285 (ε 8800) and 231 nm (16,500), $\lambda_{max.}$ (NaOH–EtOH) 301 (ε 10,500) and 247 nm (19,500), $\tau(CF_3CO_2H)$ 2·60br (2H, s, $\overset{+}{N}H_2$), 3·13—3·36 (5H, m, aromatics), 5·28 (1H, m, 1-H), 6·11 (3H, s, OMe), 6·13 (3H, s, OMe), and 5·66—6·88 (6H, complex, aliphatics), *m/e* 315 ($M^+ - HBr$), 178 (100%), 163, and 137 (Found: C, 54·5; H, 5·8; N, 3·4. $C_{28}H_{22}BrNO_4$ requires C, 54·5; H, 5·6; N, 3·5%).

The free base, had m.p. 145—147° (from EtOAc), $[\alpha]_D$ +42° (*c* 0·18 in $CHCl_3$) and was chromatographically and spectroscopically identical with an authentic sample of (±)-*N*-nororientaline. This identity was confirmed by conversion to the hydrochloride, m.p. 247—249° (from MeOH–Et_2O) (lit.,[34] m.p. 249—250°).

The free base (20 mg) in methanol (1 ml) was treated with methyl iodide (1 ml) to give crystals of the methiodide, m.p. 125—127°, $[\alpha]_D$ −57°, (*c* 0·113 in Me_2CO) and a solution which after column chromatography on alumina (Grade III), gave a product chromatographically identical with a sample of authentic orientaline (IV; $R^1 = R^4 = R^5 = Me$, $R^2 = R^3 = H$). The perchlorate had m.p. 128—130° (from Me_2CO–Et_2O) (lit.,[14] m.p. 127°).

Extraction of E. fusca *Lour.*—Powdered dried leaves of *E. fusca* Lour. (2·69 kg) were extracted as above to yield the crude alkaloidal fraction (3·90 g, 0·15%). T.l.c. analysis indicated a single basic component present in isolable quantities. This was purified by preparative t.l.c. [C_6H_6–EtOAc (4 : 1)]. The noncrystalline product (48·2 mg) in ethanol (0·5 ml) was treated with picric acid (10 mg) in ethanol (1 ml). Addition of light petroleum (1 drop) precipitated fine needles of erysotrine picrate (I; $R^1 = R^2 = R^3 = Me$, $R^4 = H$), (5 mg, 0·0002%), m.p. 159—161° (from EtOH), $[\alpha]_D$ +141° (*c* 0·16 in EtOH), [lit.,[21] m.p. 160—161°, $[\alpha]_D$ +138°], *m/e* 313 (M^+), 298, and 282 (100%), identical with an authentic sample prepared by methylation of erysovine (0·5 mg) with ethereal diazomethane in methanol (0·2 ml).[35]

Extraction of E. lithosperma *Blume.*—Powdered dried leaves of *E. lithosperma* Blume (3·95 kg) were acid extracted with 0·1N-H_2SO_4 (4·5 l) as above to yield a crude alkaloidal extract (38·8 g, 0·98%) which was chromatographed on alumina (Grade III). Elution with benzene–ethyl acetate (2 : 1) gave traces of an alkaloid with the chromatographic properties of erythraline (I; $R^1,R^2 = CH_2$, $R^3 = Me$, $R^4 = H$) together with erysotrine (I; $R^1 = R^2 = R^3 = Me$, $R^4 = H$) (8·75 g, 0·22%) which was purified as the picrate, m.p. 159—161° (from EtOH), $[\alpha]_D$ +142° (*c* 0·122 in EtOH) (lit.,[36] m.p. 160—161°, $[\alpha]_D$ +138°).

Further elution gave crude samples of erythratidinone (4·73 g, 0·12%) and 3-demethoxyerythratidinone (619 mg, 0·016%) and finally unresolved traces of a complex mixture of polar alkaloids.

Erythratidinone (II; $R^1 = R^2 = Me$, $R^3 = OMe$, $R^4,R^5 = O$).—The crude material above was crystallised to give erythratidinone, m.p. 119—120° (from C_6H_6–light petroleum), $[\alpha]_D$ +358° (*c* 1·121 in $CHCl_3$), $\nu_{max.}$ 1675 cm^{-1}, $\lambda_{max.}$ (EtOH), 231 (ε 19,600) and 284 nm (3950), τ 3·32br (1H, s, 17-H), 3·43 (1H, s, 14-H), 3·87 (1H, m, 1-H), 5·95 (1H, m, 3-H), 6·12 (3H, s, OMe), 6·22 (3H, s, OMe), 6·50 (3H, s, OMe), and 6·68—7·72 (10H, complex, aliphatics), $\tau(C_6D_6)$ 3·40br (1H, s, 17-H), 3·62 (1H, s, 14-H), 1·08 (1H, m, 1-H) 5·98 (1H, q, $J_{3,4e} + J_{3,4a}$ 17·5 Hz, 3-H), 6·54 (3H, s, OMe), 6·62 (3H, s, OMe), 6·66 (3H, s, OMe), and 6·92—7·92 (10H, complex, aliphatics), *m/e* 329 (M^+), 301, 298, 286, 272, 271 (100%), 243, 242, 228, 215, 214, and 197 (Found: C, 69·0; H, 6·8; N, 4·2. $C_{19}H_{23}NO_4$ requires C, 69·3; H, 7·0; N, 4·3%). The free base was converted into the picrate, m.p. 205—207° (from EtOH), $\nu_{max.}$ (Nujol), 1698 cm^{-1}.

Erythratidine (II; $R^1 = R^2 = Me$, $R^3 = MeO$, $R^4 = H$, $R^5 = OH$) *and Epierythratidine* (II; $R^1 = R^2 = Me$, $R^3 = MeO$, $R^4 = OH$, $R^5 = H$).—Erythratidinone (696 mg) in methanol (10 ml) was reduced with sodium borohydride (1 g) during 2·5 h. Aqueous work-up and chloroform extraction gave a crude product which was separated by p.l.c. on alumina GF to give erythratidine (379 mg, 54%), m.p. 120—120·5° (from EtOAc–light petroleum), $[\alpha]_D$ +258° (*c*, 0·581 in $CHCl_3$), $[\alpha]_D$ +273° (*c* 0·109 in EtOH) [lit.,[23] m.p. 120—121°, $[\alpha]_D$ +279° (in EtOH)], $\nu_{max.}$ 3509 and 3387 cm^{-1}, $\lambda_{max.}$ 219 (ε 22,500), 232infl (5700), and 284 nm (2600), τ 3·42br (1H, s, 17-H), 3·54 (1H, s, 14-H), 4·19 (1H, m, $J_{1,2e}$ 4·2 Hz, 1-H), 5·57 (1H, m, $J_{2e,3e}$ 4·2 Hz, 2-H), 6·40 (1H, m, 3-H), 6·20 (3H, s, OMe), 6·26 (3H, s, OMe), 6·70 (3H, s, OMe), and 6·20—8·32 (11H, complex, aliphatics), *m/e* 331 (M^+), 300, 273, 257 (100%), and 244 (Found: C, 68·8; H, 7·8; N, 4·2. Calc. for $C_{19}H_{25}NO_4$: C, 68·9; H, 7·6; N, 4·2%). The base gave a picrate, m.p. 220—222° (from Me_2CO) (lit.,[23] m.p. 222—224°).

Also isolated from the p.l.c. was *epierythratidine* (166 mg, 24%), m.p. 67—68° (from EtOAc–light petroleum), $[\alpha]_D$ +142° (*c* 0·148 in $CHCl_3$), $\nu_{max.}$ 3574 and 3427 cm^{-1}, τ 3·20 (1H, s, 14-H), 3·42 (1H, s, 17-H), 4·40 (1H, m, 1-H) 5·50 (1H, m, 2-H), 5·03 (6H, s, OMe), 6·43 (1H, m, 3-H), 6·70 (3H, s, OMe, and 6·03—8·33 (11H, complex, aliphatics).

[32] V. Boekelheide and M. F. Grundon, *J. Amer. Chem. Soc.*, 1953, **75**, 2563.
[33] V. Boekelheide, J. Weinstock, M. F. Grundon, G. L. Sauvage, and E. J. Agnello, *J. Amer. Chem. Soc.*, 1953, **75**, 2550.
[34] M. Tomita and J. Kunitomo, *J. Pharm. Soc. Japan*, 1960, **80**, 1238.
[35] R. A. Labriola, V. Deulofeu, and B. Berinzaghi, *J. Org. Chem.*, 1951, **16**, 90.
[36] F. Koniuszy, P. F. Wiley, and K. Folkers, *J. Amer. Chem. Soc.*, 1949, **71**, 875.

The mass spectrum was identical with that of erythratidine (Found: M^+, 331·1788. $C_{19}H_{25}NO_4$ requires M, 331·1783).

Dehydration of Erythratidine (II; $R^1 = R^2 = Me$, $R^3 =$ OMe, $R^4 = H$, $R^5 = OH$).—Erythratidine (103 mg) in dry pyridine (2 ml) was treated with methanesulphonyl chloride (0·5 ml) in dry pyridine (1 ml) at 0° during 1 h. The mixture was diluted with water (40 ml) adjusted to pH 8 with sodium carbonate solution, and extracted with chloroform (5 × 10 ml). The extract was dried (Na_2SO_4) and evaporated and the residue was separated by p.l.c. to give (in minor amount) erysotrine, chromatographically identical with authentic material, together with the *2-chloro-derivative* (II; $R^1 = R^2 = Me$, $R^3 = OMe$, R^4 or $R^5 = Cl$, R^5 or $R^4 = H$) (19·6 mg 19%), m.p. 150—152° (from C_6H_6–EtOAc), τ 3·28 (1H, s, 14-H), 3·45 (1H, s, 17-H), 4·37 (1H, m, 1-H), 5·47 (1H, m, 2-H), 6·00 (6H, s, OMe), 6·53 (3H, s, OMe), and 6·00—7·93 (11H, complex, aliphatics), *m/e* 349 (M^+), 314, 313, 298, 293, 291, 282, 258, 257 (100%), and 256 (Found: M^+, 349·1450. $C_{19}H_{24}ClNO_3$ requires M, 349·1445).

3-Demethoxyerythratidinone (II; $R^1 = R^2 = Me$, $R^3 =$ H, R^4,$R^5 = O$).—The crude alkaloidal fraction (135 mg) was purified by p.l.c. on alumina GF to give *3-demethoxy-erythratidinone* (91 mg), m.p. 111—112° (from C_6H_6–light petroleum), $[\alpha]_D$ +325° (*c* 0·249 in $CHCl_3$), $\lambda_{max.}$ 231 (ε 15,300) and 284 nm (35,050), $\nu_{max.}$ 1667 cm^{-1}, τ 3·41br (1H, s, 17-H), 3·49 (1H, s, 14-H), 3·96 (1H, m, 1-H), 6·21 (3H, s, OMe), 6·32 (3H, s, OMe), and 6·48—8·15 (12H, complex, aliphatics), *m/e* 299 (M^+), 272, 271 (100%), 243, 242, 222, 215, 214, 212, and 197 (Found: C, 72·1; H, 7·1; N, 4·6. $C_{18}H_{21}NO_3$ requires C, 72·2; H, 7·1; N, 4·7%). The base was converted into the picrate, m.p. 200—202° (from Me_2CO–MeOH), (Nujol) 1680 cm^{-1}.

We thank the Gulbenkian Foundation and the British Council for financial support (to A. M. F. T. L. and A. A. L. G. respectively), Professor A. H. Jackson for (±)-isoboldine and (+)-glaucine, and Dr. D. S. Bhakuni for (±)-*N*-nororientaline. For the generous supplies of plant material we thank Dr. P. R. Enslin (*E. lysistemon*), Dr. S. Flores (*E. peoppigiana*), and Professor M. U. S. Sultanbawa (*E. fusca* and *E. lithosperma*).

[2/2649 *Received*, 22*nd November*, 1972]