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ALKALOIDS OF *COCCULUS LAURIFOLIUS* D.C.

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Abstract Ten new abnormal *Erythrina* alkaloids, isococculidine, isococculine, coccuvine, coccuvinine, cocculitine, cocculitinine, coccudinone, cocculimine, coccudienone, coccoline and coccolinine have been isolated from the leaves of *Cocculus laurifolius* D.C. and their structures and stereochemistry is assigned by chemical transformations and spectral studies. The known proaporphine alkaloid stepharine and the morphinandienone alkaloid, sebiferine have also been isolated for the first time from the plant.

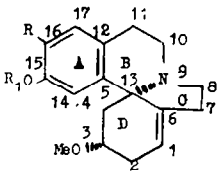
Cocculus laurifolius D.C. (menispermaceae) an evergreen shrub grows in tropical and subtropical regions of the world and has been extensively investigated for its alkaloidal constituents.[1-10] Confirmation of hypotensive and neuromuscular blocking activities in the 50% ethanolic extract of *C. laurifolius*[11] during a programme aimed at screening Indian plants over a wide range of biological activities prompted its reinvestigation which resulted in the isolation of several abnormal *Erythrina*, dibenz [d,f] azonine, proaporphine, quaternary aporphine, morphinandienone and simple 1-benzyltetrahydroisoquinoline alkaloids. Preliminary reports on the structures, of some of these bases have been communicated earlier. A fuller account of the work leading to these structures and information regarding the structures of other alkaloidal constituents of the plant are now presented.

The alkaloids from the alkaloidal mixture, obtained from the ethanolic extract of stems and roots of *C. laurifolius* were isolated by acid–alkali treatment, solvent fractionation, extensive column and preparative tlc on. The isolated abnormal *Erythrina* alkaloids are: cocculine[7] (**1**), cocculidine[7] (**2**), dihydroerysodine[9] (**3**), isococculidine[12] (**5**), isococculine[13] (**6**), coccuvine[14] (**7**), coccuvinine[15] (**8**), cocculitine[16] (**9**), cocculitinine (**10**), cocculidinone (**11**), cocculimine (**12**), coccoline[12] (**13**), coccolinine[17] (**14**) and coccudienone (**15**). The dibenz (d, f) azonine alkaloids laurifonine[18] (**19**), laurifine[18] (**20**) and laurifinine[18] (**21**), the morphinandienone alkaloid, sebiferine[19] (**16**), the proaporphine alkaloids stepharine[20] (**17**) and N-methylstepharine[20] (**18**), the quaternary aporphine alkaloids magnoflorine,[4] laurifoline,[9] chlorides isocorydine, O-methylisocorydine,[21] and boldine, methochlorides and the simple 1-benzyltetrahydroisoquinoline alkaloids coclaurine, N-methylcoclaurine, reticuline and laudanidine. Of the isolated bases the *Erythrina* alkaloids, cocculine[7] (**1**), cocculidine[7] (**2**), dihydroerysodine[8] (**3**), the quaternary aporphine alkaloids magnoflorine[4] and laurifoline[9] and the simple benzylisoquinoline alkaloids coclaurine[1], N-methylcoclaurine[1], reticuline[2], and laudanidine have been isolated earlier from *C. laurifolius*. The alkaloids isolated from other sources but isolated for the first time from the plant are sebiferine[19] (**16**), stepharine[21] (**17**) and N-methylstepharine[21] (**18**).

Cocculine (**1**) and cocculidine (**2**) the first member of abnormal *Erythrina* alkaloids were isolated by Yunusov.[7] The structure and configuration of these bases have been established unequivocally by spectral and X-ray crystallographic data and confirmed by chemical studies.[22] The spiro structures of **1**, and **2**, was shown by formation of the NO-diacetyl derivative (**4**) with acetic anhydride. The NMR spectra of **1** and **2** showed that these bases are related to the *cis* series of $\Delta^{1(6)}$ *Erythrina* alkaloids.[23]

The abnormal *Erythrina* alkaloid isococculine (**6**) on treatment with diazomethane gave isococculidine. Isococculidine was thus the O-Me derivative of isococculine. The IR and UV spectra of these bases are very similar to that of 1,2-diene type of *Erythrina* alkaloids.[24] The NMR spectrum of isococculine (**6**) was almost identical with isococculidine (**5**) except that a signal for an aryl OMe function present at 6.2 in the NMR spectrum of **5** was absent in the spectrum of **6**. A three proton signal for an aliphatic OMe group in **6** resonated at τ 6.76. Of the three aromatic protons one *meta*-coupled proton was centred at 3.26 (1 H, J = 2.00 Hz) and one *meta*- and *ortho*-coupled proton appeared at τ 3.33 (1 H, dd, $J_1 = 8$; $J_2 = 2.0$ Hz) and one *ortho*-coupled proton was centred at τ 2.3 (1 H, d, J = 8 Hz). Two olefinic proton multiplets are at τ 3.92 and 4.17. Double resonance experiments gave the coupling constants for the ring 'D' protons ($J_{1,2} = 10.5$; $J_{2,3} = 1.5$; $J_{3,4a} = 10.0$; $J_{3,4e} = 6$; $J_{4a,4e} = 12$; $J_{1,3} = 1.5$; $J_{1,6} = 3.5$ and $J_{2,6} = 1.5$ Hz). Assuming the basic erythrinan structure for isococculine the coupling of the proton α- to the OMe group (H-3), to an olefinic proton fixed the position of the double bond at 1(2) and the magnitude ($J_{2,3} = 1.5$ Hz) indicated the 4-axial conformation of H-3. The OMe group must, therefore, be equatorial as in the previously characterised alkaloids. The coupling of H-3 to H-4a (10 Hz) and H-4e (6 Hz) confirmed this assignment. A value for $C_{1,6}$ of 3.5–4 Hz indicated a equatorial orientation for H-6. The OH group present in isococculine was placed at position δ 15 as follows: irradiation at τ 7.10 (benzylic region) sharpened a low field doublet at τ 2.92 due to an *ortho*-coupled aromatic proton. There was no effect on the other aromatic protons, irradiation 10.0 Hz either side τ 7.12 had no effect.

The mass spectra of isococculidine (**5**) and isococculine (**6**) are in complete agreement with the



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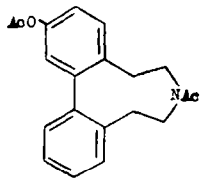
(2) R=H; R$_1$=Me
(3) R=OH, R$_1$=Me



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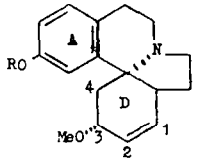
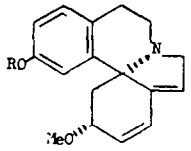


(5) R=Me
(6) R=H



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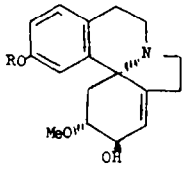
(8) R=Me



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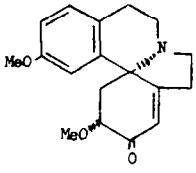

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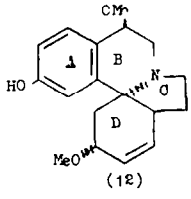

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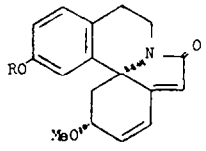

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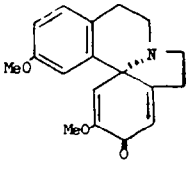
(14) R=H



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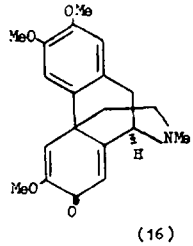

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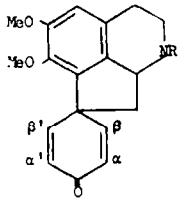
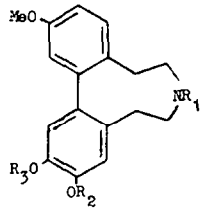
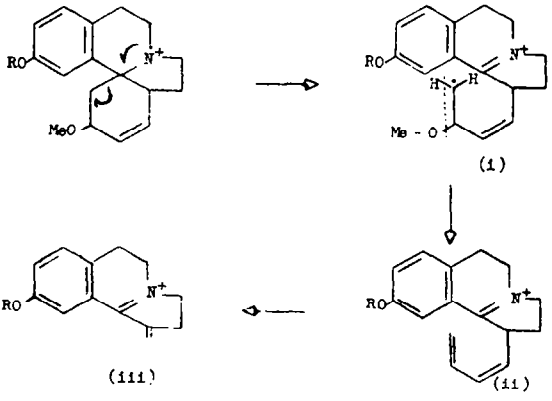


(17) R=H
(18) R=Me



(19) R$_1$=R$_2$=R$_3$=Me
(20) R$_1$=H; R$_2$=R$_3$=Me
(21) R$_1$=R$_3$=Me, R$_2$=H



(1)
(ii)
(iii)

Scheme 1. Mass spectral fragmentation of isococculidine (5) and isococculine (6).

proposed structures. The prominent ions in the spectra were at *m/e* $M^{\cdot}$, $M^{\cdot}-15$, $M^{+}-31$ (base peak), $M^{+}-59$, $M^{+}-73$ and $M^{+}-85$. A rationale of fragmentation pattern of isococculidine and isococculine is given in Scheme 1.

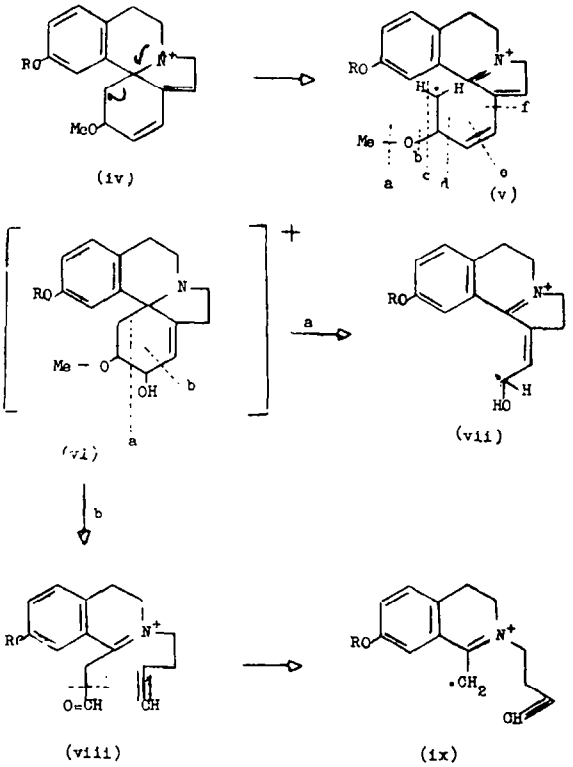
The IR and UV spectra of coccuvine (**7**) (ν_{max}: 3450 and 1610 cm^{-1} and λ_{max}: (EtOH) 228 and 284 nm; λ_{max}: (EtOH–NaOH) 308 nm) and coccuvinine (**8**) (ν_{max}: 1603 cm^{-1} and λ_{max} (EtOH) 228 and 282 nm, no change in NaOH) were suggestive of aromatic *Erythrina* alkaloids having a 1,6-diene system.[24] The NMR spectra of coccuvine (**7**), and coccuvinine (**8**) are almost identical with erysotrine[24] and erythraline.[24] The only apparent difference being in the number of signals for OMe and methylenedioxy groups. In coccuvine (**7**) there was no signal for an aryl OMe function. In the spectrum of coccuvinine (**8**) the signal for an aromatic OMe group was at τ 6.72. In the spectra of both the bases, there were signals for 3 aromatic and 3 olefinic protons. One *meta*-coupled aromatic proton was centred at τ 3.22 (1 H, S, J = 2.0 H_2), one *meta*- and *ortho*-coupled proton was at τ 3.30 (1 H, dd, J_1 = 8, J_2 = 2.0 Hz) and one *ortho*-coupled proton was centred at τ 2.96 (1 H, d, J = 8 Hz). The 3 olefinic protons forming an ABX system appeared at τ 3.45 (1 H, dd, J_1 = 10.0 and J_2 = 2.0 Hz), 4.04 (1 H, dd, J_1 = 10.0 and J_2 = 0.5 Hz) and at τ 4.28 (1 H, S). Irradiation at τ 6.50 (α-to oxygen) caused a collapse of the small (2 Hz) splitting of the olefinic protons at τ 3.45, leaving the AB system (J = 10 Hz) of the two lower field olefinic protons. This infers a *cis*-orientation of the double bond. The irradiation also sharpened the signal at τ 4.02 of the 'A' part of the system indicating 0.5 Hz allylic coupling. These results are accommodated by the 3-methoxy-1,6-diene system of the *Erythrina* alkaloids and imply a 3 4 equatorial conformation for the methoxyl group, as is present in the previously characterised *Erythrina* alkaloids.[25,26]

Treatment of coccuvine (**7**) with CH_2N_2 gave coccuvinine (**8**). Which is therefore an OMe derivative of **7**. The aryl OMe group in coccuvinine (**8**) was placed at position 15 as follows: irradiation at τ 7.09 (benzylic region) sharpened a lowfield doublet at τ 2.96 due to an *ortho*-coupled aromatic proton. There was no effect on the orther aromatic protons. Irradiation 10.0 Hz either side of τ 7.12 had no effect.

The mass spectra of coccuvinine (**8**) and coccuvine (**7**) are in complete agreement with the proposed structures. The prominent ions in the spectra were at *m/e* $M^{\cdot}$, $M^{+}-15$, $M^{\cdot}-31$ (base peak), $M^{+}-58$, $M^{+}-60$, $M^{\cdot}-71$ and $M^{\cdot}-84$. A rationalization of this based on the established precedent[27] is given in (V).

Reduction of coccuvinine (**8**) and coccuvine (**7**) separately in methanol with 10% Pd/C afforded dihydro derivatives identical in all respects with cocculidine[22] (**2**) and cocculine[22] (**1**) of established stereochemistry.

The IR spectrum (ν_{max}: 3460 cm^{-1}) of cocculitinine (**10**) indicated the presence of an OH group. UV spectrum (λ_{max} 283; λ_{max} (EtOH–NaOH) 307 nm) suggested that the OH group in the base is phenolic in nature. Treatment of cocculitinine (**10**) with CH_2N_2 gave an OMe derivative identical with cocculitine (**9**). The treatment of **9** with Ac_2O/pyridine formed a mono acetate. NMR spectra of cocculitine and cocculitinine were comparable with that reported for erythratine[25] except that the methylenedioxy group of erythratine was replaced by an aromatic OMe group signal at τ 6.24. In the spectrum of cocculitinine there was no signal for an aromatic OMe group. An aliphatic OMe group present in both the bases resonated at about τ 6.72. The aromatic region had signals for 3 protons. The orientation of these protons was the same as that of other abnormal *Erythrina* alkaloids. The signal at τ 4.36 due to an olefinic proton was rather complex but on irradiation at τ 5.70 it gave an ill defined triplet splitting and irradiation at τ 7.75 gave a sharp doublet with $J_{1,2}$ = 3.0 Hz. The small triplet splitting was due to allylic coupling between the C-1 and C-7 protons. Of the three oxygen functions in cocculitine (**9**) two were involved in ether linkages and the remaining one was engaged in an alcoholic group as was shown by the formation of mono acetyl derivative. One proton signal at τ 5.64 present in the spectrum of cocculitine moved to τ 4.50 in the spectrum of its acetyl derivative confirming that it was due to the proton on the carbon carrying an OH function. The relative stereochemistry at different centers in cocculitine as shown in **9** was established by double resonance technique and by comparison of the NMR data with that of erythristemine,[25] the X-ray structure of which has been determined and with erythratine[28] of known stereochemistry. The C-2 and C-3 protons showed a *trans*-diaxial coupling ($J_{2,3}$ = 8.5 Hz). The mass fragmentation of cocculitine (**9**) and cocculitinine (**10**) confirmed the position of the ethylenic bond at $\Delta^{1(6)}$ since a major peak in the mass spectra of these bases were at *m/e* $M^{\cdot}-58$ corresponding to reverse Diels Alder type of fragmentation. A rationalisation of the formation of other prominent ions based on established precedent[25] is given in Scheme 2.



Scheme 2 Mass spectral fragmentation of cocculitine (9) and cocculitinine (10).

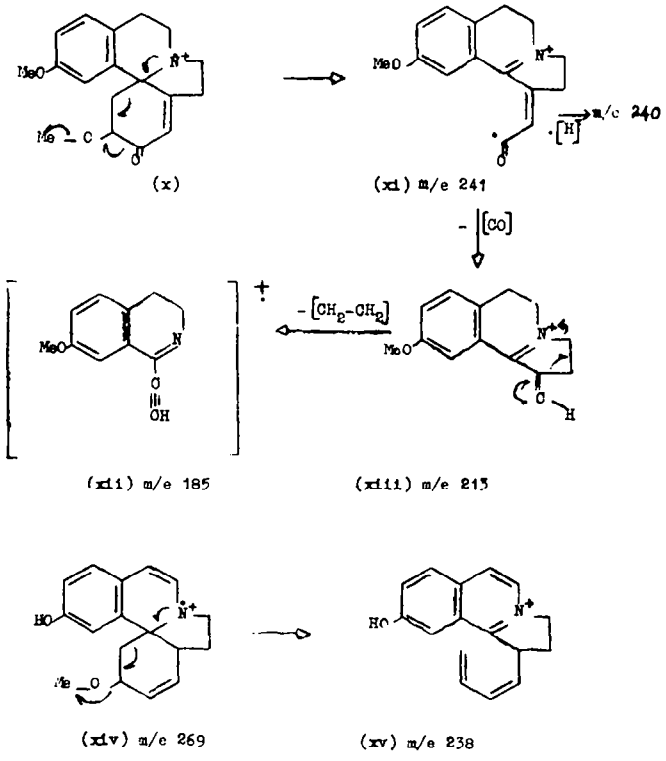
Oxidation of cocculitine (**9**) gave coccudinone (**11**) which was reduced to give a mixture of cocculitine (**9**) and epicocculitine. The IR spectrum (dilute CCl_4 solns) of epicocculitine and cocculitine showed OH absorption bands at ν_{max} 3558 and 3609 cm^{-1} respectively. The strong H-bonding in epicocculitine is consistent[29] only with *cis*-arrangement of the OH and OMe groups. Cocculitine must, therefore, be the *trans*-isomer. The stereochemistry at the spiro-centre in the base was defined by conversion of cocculitine with methanesulphonyl chloride in pyridine into coccuvinine (**8**). Application of Mills rule[30] to the optical rotations of cocculitine and epicocculitine confirms the configuration of cocculitine as in **9**.

The IR (ν_{max}: 1662 and 1610 cm^{-1}) and UV spectra (λ_{max}: 230 and 282 nm) of cuccudinone (**11**) was suggestive of the presence of an enone system. In the NMR spectrum of the base there were signals for an aromatic and an aliphatic OMe groups at τ 6.24 and 6.67 respectively. The trisubstituted olefinic proton at C_1-H was coupled with C_7-H and C_3-H and forming an quartet. There were three protons in the aromatic region and their orientation was that of other abnormal *Erythrina* alkaloids.

The mass fragmentation of coccudinone (**11**) is in complete agreement with the proposed structure. The characteristic ions were *m/e* 299 (M^+), 284 (M^+-15), 255 (M^+-44), (base peak), 241 (M^+-58), 240 (M^+-59), 231 (M^+ 86) and 185 (M^+-114). A number of these peaks were related by metastable ions. A mechanistic interpretation of the formations of these ions based on initial retro- Diels- Alder type fragmentation[27] is given in Scheme 3.

Reduction of coccudinone (**11**) gave a mixture of cocculitine (**9**) and epicocculitine. Treatment of the mixture of epimeric alcohols with methanesulphonyl chloride afforded coccuvinine (**8**), albeit in poor yield. The stereochemistry at the spiro centre in coccudinone (**11**), was thus correlated with that of coccuvinine (**8**).

The IR (ν_{max} (KBr) 3468 and 1600 cm^{-1}) and UV spectra (λ_{max}: 230 and 284 nm and λ_{max} (EtOH-NaOH) 240 and 298 nm) of cocculimine (**12**) were very similar to that of 1,2-diene type of *Erythrina* alkaloids.[12] Cocculimine formed an acetyl derivative. The NMR spectrum of the base was almost identical with that of isococculine[13] (**6**) except that it had an additional signal for an aliphatic OMe group at τ 6.70 and a multiplet at τ 6.06 for a proton. The aliphatic OMe group at C-3 appeared at τ 6.76. Of the three aromatic protons one *meta*-coupled proton was centred at τ 3.24 (1 H, d, J = 2.00 Hz) and one *meta*- and *ortho*-coupled proton was at τ 3.28 (1 H, dd, J_1 = 8; J_2 = 2.0 Hz) and one *ortho*-coupled proton was centred at τ 2.96 (1 H, d, J = 8 Hz). Two olefinic proton multiplets were at τ 3.94 and 4.20. Double resonance experiments gave the coupling constants for the ring 'D' protons as: ($J_{1,2}$ = 10.5; $J_{2,3}$ = 1.5; $J_{3,4a}$ = 10.0; $J_{3,4e}$ = 6; $J_{4,4e}$ = 12.0, $J_{1,3}$ = 1.5; $J_{1,6}$ = 3.5 and $J_{2,6}$ = 1.5 Hz). Assuming the basic erythrinan structure for cocculimine the coupling of the proton α- to the OMe group (H-3) to an olefinic proton fixed the position of the double bond at 1,2 and the magnitude ($J_{2,3}$ = 1.5 Hz) indicated the 4-axial conformation of H-3. The OMe group must, therefore, be equatorial as in the previously characterised alkaloids.[13,14] The coupling of H-3 to H-4a (10 Hz) and H-4c (6 Hz)



Scheme 3. Mass spectral fragmentation of coccudinone (**11**).

confirmed this assignment. A value for $J_{2,6}$ of 3.5–4 Hz indicated a equatorial orientation for H-6. Irradiation at τ 6.06 (benzylic region) sharpened a low field doublet at τ 2.96 due to an *ortho*-coupled aromatic proton. There was no effect on the other aromatic protons. This fixed the position of aromatic OH group at C-15. Irradiation of the aromatic signal at τ 2.96 (17-H) caused a slight narrowing of the signal at τ 6.06 which suggested that this was the benzylic proton at C-11. The protons at C-10 and C-11 were obscured to some extent by the OMe signals. The configuration of 11-OMe of cocculimine (**12**), however, remains undefined.

The prominent ions in the mass spectrum of cocculimine (**12**) were ions (XIV and XV) *m/e* 269 (base peak) and 238 respectively. The other significant peaks in the spectrum were at *m/e* 301 (M^+), 286 (M^+-15), 268 (M^+-33) and 254 (M^+ 47). The fragmentation pattern was in agreement with the proposed structure.

The IR (ν_{max} (KBr) 3462 cm^{-1}) and UV spectra (λ_{max} 230, 256 and 284 nm and λ_{max} (EtOH–NaOH) 302 nm) of coccoline (**14**) indicated the presence of a phenolic OH group. In the IR spectrum of coccolinine (**13**) a strong CO absorption band was at 1665 cm^{-1} and there was no absorption in OH region. The UV spectrum of the compound had absorptions peaks at 231, 258 and 284 nm, which remained unchanged in presence of NaOH. Treatment of coccoline (**14**) with diazomethane afforded an OMe derivative identical with coccolinine (**13**) which is therefore an OMe derivative of coccoline (**14**). The UV absorption peak at 256 nm exhibited by these compounds was not normally present in *Erythrina* alkaloids and taken with the 1665 cm^{-1} absorption band in the IR spectrum suggested the presence of a dienone system. The NMR spectra of coccoline, and coccolinine were almost identical except that a signal for an aromatic OMe function present in the spectrum of the former was absent in the latter. In both the compounds a three proton singlet at about τ 6.70 was for an aliphatic OMe group. In the aromatic region there were three protons. One *meta*-coupled proton was centred at τ 3.30 (1 H, d, J = 2 Hz) and one *meta*- and *ortho*-coupled proton was at τ 3.28 (1 H, dd, J_1 = 8; J_2 = 2 Hz) and an *ortho*-coupled proton was at τ 2.96 (1 H, d, J = 8 Hz). The three olefinic protons were forming an ABX system. A low field signal at τ 3.42 comprising the 'B' component of the ABX system, a signal at τ 3.85 comprising the 'A' component of the same system and one at τ 4.16 (singlet). Irradiation at τ 6.50 (α- to oxygen) caused a collapse of the small (2 Hz) splitting of the olefinic protons at τ 3.42 leaving the AB system (J = 10 Hz) of the two lower field olefinic protons. This implies a *cis*-orientation of the double bond. The irradiation also sharpened the signal of the 'A' part of the system (τ 3.85) indicating 0.5 Hz allylic coupling. These results are accommodated by the 3-methoxy-1,6-diene system of the *Erythrina* alkaloids. However, the lack of fine structure of the remaining olefinic proton, the low field of the double doublets as well as the IR and UV spectra require that the CO group be placed at C-8. The coupling constant between H-3 and H-2 (J = 2 Hz) was comparable to that of other *Erythrina* alkaloids.

The mass fragmentation patterns of coccoline and coccolinine are consistent with the proposed structures 13 and 14 respectively. The molecular ion (M^+) was the base peak. In particular there were intense peaks at *m/e* M^+-15, M^+-31, M^+-59 and M^+-61. The low intensity peaks were at *m/e* M^+-29, M^+ 43 and M^+-87. The absolute stereochemistries of coccolinine (**13**) and coccoline (**14**) have not been determined but were assigned on the basis of that determined for cocculidine[22] (**2**) and cocculine[22] (**3**). Coccoline and coccolinine are perhaps artifects and may be formed from coccuvinine (**8**) and coccuvine (**7**) respectively during the drying process.

The IR (ν_{max}: 1670, 1650 and 1625 cm^{-1}) and UV (λ_{max} 242 and 283 nm) spectra of coccudienone (**15**) were suggestive of the presence of dienone system in the base. The NMR spectrum of the compound was almost identical with that of erysodienone.[31] In the spectrum of coccudienone (**15**) there were 3 aromatic protons and these appeared at τ 2.92 (1 H, dd, J = 8.0; J_2 + J benzylic = 3 Hz, H-17), 3.32 (1 H, dd, J_1 = 8.0, J_2 = 2.5 Hz), H-16) and 3.68 (1 H, brs, H-14). Two OMe groups were at τ 6.30 (3 H, S, 15 OMe), 6.39 (3 H, S, 3–O–Me) and 2 olefinic protons at τ 3.74 (1 H, t, J = 2.0 Hz, H-1) and 4.04 (1 H, S, H-4). In the mass spectrum the significant peaks were at *m/e* 297 (M^+), 282 (M^+-15), 266 (M^+-31) (base peak) and 218 (M^+-78).

The IR (λ_{max} 1660, 1640 and 1620 cm^{-1}) and UV (λ_{max} 238 and 282 nm) spectra of sebiferine (**16**) suggested the presence of a cross-conjugated cyclohexadienone system and supported by its mass spectrum *m/e* 341 (M^+), 236 (M^+ 15), 313 (M^+ 28), and 298 (M^+-41). In the NMR spectrum the signals were at τ 7.58 (NMe), 6.23 (OMe), 6.18 (OMe) and 6.15 (OMe). There were two olefinic protons at τ 3.28 (1 H, S, 8-H) and 3.75 (1 H, S, 5-H) and 2 aromatic protons at τ 3.64 (1 H, S) and 3.40 (1 H, S). The m.p., IR, UV, NMR and MS of the compound were almost identical with (±)-O-methylflavinantine[32] and with an alkaloid from *Litsea sebifera*.[19] However, direct comparison with authentic sample could not be possible. The absolute configuration of sebiferine as shown in **16** has been determined.[33]

The IR (ν_{max}: 3200 (NH), 1655, 1620 and 1602 cm^{-1}) and UV spectra (λ_{max}: 236 and 285 nm) of stepharine was suggestive of a cross conjugated dienone system. In the NMR spectrum the signals for one aromatic proton and two aromatic OMe groups were at τ 3.30 (1 H, S, 3-H), 6.28 (3 H, S, OMe) and 6.17 (3 H, S, OMe). 4 Olefinic protons forming multiplets were centred at τ 2.9 and 3.8. The multiplets were arising from the two overlapping AB quartets from the β and α protons of an unsymmetrical 4,4′-disubstituted cyclohexa-2,5-dienone. Transannular coupling $J_{\alpha\alpha'}$, 1.5 and $J_{\beta\beta'}$ = 2.5 Hz) of the α,α′ and β protons was also present. In the mass spectrum of the base the significant ions were at *m/e* 297 (M^+), 296 (M^+-1), 268 (M^+-29), 253 (M^+-44), 237 (M^+-60) and 225 (M^+-72). Treatment of the base with formaldehyde formic acid gave N-Me derivative identical with N-methylstepharine.[20]

Of the various bases isolated from *C. laurifolius*, isococculidine (**5**) the major alkaloid of the leaves of the plant was found to have neuromuscular blocking and hypotensive activities.[34] Cocculidine (**2**) and cocculine (**1**) isolated earlier and by us also from the leaves of the plant have hypotensive activity.[35] This activity in these bases was due to ganglionic blocking action. The quaternary aporphine alkaloids

magnoflorine and laurifoline, chlorides, O-methylisocorydine, isocorydine boldine, methochlorides exhibited *d*-tubocurarine like curarising action on sciatic skeletal muscles. These quaternary bases also induced hypotensive effects in dogs, cats and rabbits. This activity was found due to considerable ganglionic blocking action of these bases on various sympathetic and *para*-sympathetic ganglia.[36]

We have studied the biosynthesis of abnormal *Erythrina* alkaloids isococculidine[37] (**5**), cocculidine[38] (**2**) and cocculine[38] (**1**) and morphinandienone alkaloids sebiferine[39] (**16**) in *C. laurifolius* and have demonstrated that the alkaloids **1**, **2** and **5** are stereospecifically biosynthesised from (+)-norprotosinomenine. O-Demethylation is the terminal step in the biosynthesis of these bases. Isococculidine (**5**) is converted into cocculidine (**2**) with very high efficiency. Sebiferine (**16**), the morphinandienone alkaloids is biosynthesised in the plants specifically from reticuline.

EXPERIMENTAL

Unless otherwise stated UV spectra refer to soln in EtOH, IR spectra to KBr and NMR spectra to solns in $CDCl_3$. Tlc was carried out, unless specified to contrary, on silica gel G.F. 254. The NMR spectra (τ scale) were taken on a varian 60 MHz or Perkin Elmer R-32 (90 MHz) instruments using HMDS as an internal standard. The mass spectra were recorded on JEOL JMS-D300 mass spectrometer fitted with a direct inlet system. All the compounds gave satisfactory elemental analysis (C, ±0.3; H, ±0.2%).

Extractions of leaves. Air dried leaves (40 mg) of *C. laurifolius* collected in September from Dehra Dun, India, were pulverised and percolated with EtOH (6 × 60 l). The solvent from the combined percolate was removed under reduced pressure below 40° to give a dark green viscous mass (4.0 g) which was extracted with 5% AcOH (6 × 1 l). The total acidic soln was defatted with light petroleum ether and then basified with Na_2CO_3 to pH 9. The liberated bases were extracted with $CHCl_3$, washed with water, dried (Na_2SO_4), and solvent removed to afford the crude alkaloidal mixture (150 g).

Separation of phenolic and non-phenolic bases. The preceding alkaloidal mixture (150 g) was extracted with ether. The ethereal soln was extracted with 5% HCl. The ether layer contained mostly non-basic material. The aqueous acidic extract containing the basic material was basified (pH 9-10) with NaOHaq and extracted with ether. The ether extract was concentrated to afford the non-phenolic bases (A) (45 g). The aqueous alkaline solution was adjusted to pH 7 with ammonium chloride and the liberated bases were extracted with $CHCl_3$, washed with water, dried (Na_2SO_4) and evaporated to give the phenolic bases (B) (35 g).

Chromatography of the mixture (A) of non-phenolic bases. The mixture (A) of the non-phenolic bases (45 g) was chromatographed over a column of neutral alumina (2 kg). The column was eluted with hexane, hexane-C_6H_6 (1:1), C_6H_6, C_6H_6-$CHCl_3$ (3:1, 1:1 and 1:3), $CHCl_3$ and $CHCl_3$-MeOH containing an increasing proportion of the polar solvent. Elution was monitored by tlc. Fractions (400 × 200 ml) were collected.

Isococculidine (**5**). The mixture of bases (10.7 g) obtained from the chromatographic fractions, eluted with C_6H_6-hexane (1:1 and 1:2) of non-phenolic alkaloidal mixture was rechromatographed on neutral alumina (400 g). The column was eluted with hexane, hexane-C_6H_6 and $CHCl_3$ mixtures (tlc control). Elution with C_6H_6-hexane (1:1) and (1:2) afforded[12] **5** (7.5 g), m.p. 95-96° (from C_6H_6, hexane), $[\alpha]_D$ + 124° (*c*, 1.2 in MeOH).

Cocculidine (**2**). Elution of the column with $CHCl_3$ gave **2**, (3.5 g) m.p. 86-87°; $[\alpha]_D$ + 260° (lit[5] m.p. 86-87°, $[\alpha]_D$ + 250.9°), spectroscopically identical in all respects with the reported material.

Cocculine (**14**). The mixture (1.8 g) obtained from $CHCl_3$-C_6H_6 (1:1) elution was rechromatographed on neutral alumina (200 g). Elution with C_6H_6-EtOAc (1:1) and EtOAc furnished *cocculine*[12] (450 mg) m.p. 245-246° (from EtOAc); $[\alpha]_D$ + 233° (*c*, 1.08 in MeOH).

Coccuvinine (**8**). Elution of the column with C_6H_6-hexane (3:1) gave a mixture (1.4 g) containing mainly **5** and **2**. The minor component was **8**. Repeated chromatography of the mixture failed to give a pure sample of coccuvinine. The mixture was then subjected to plc (plates: silica gel; solvent: $CHCl_3$-MeOH, 90:10) which yielded pure *coccuvinine*[15] (93 mg) m.p. 103-104° (hexane), ν_{max}: 2941, 1603, 1497, 1285 and 1101 cm^{-1}, λ_{max} (MeOH) 228 and 282 nm; λ_{max} (MeOH-NaOH) no change.

Coccudinone (**11**). Elution with C_6H_6 gave a mixture which was subjected to plc (plates: silica gel, solvent: EtOAc-C_6H_6, (1:1) to give *coccudinone* **11** as an oil, λ_{max}: 230 and 280 nm; ν_{max} (neat) 2820, 1665, 1600, 1245 and 1110 cm^{-1}; *m/e* 299 (M^+), 284 (M^+-15), 268 (M^+-31), 254 (M^+-45), 241 (M^+-90) and 208 (M^+-91).

Coccudienone (**15**). The mixture from which coccudinone had been isolated was again subjected to plc (three times) on silica gel plates (solvent: EtOAc-C_6H_6, 3:1, 1:1) to give **15** (27 mg) as amorphous powder; ν_{max}: 1660, 1640 and 1615 cm^{-1}; λ_{max} 242 and 283 nm.

Sebiferine (**16**). C_6H_6-$CHCl_3$ (1:1) elution gave a mixture which was applied to plc on silica gel plates (solvent: $CHCl_3$-MeOH, 93:7) to give **16** (320 mg) m.p. 112-113° (MeOH); $[\alpha]_D$ + 16.09° (*c*, 1.02 in MeOH) (lit[39] 112-113°).

Cocculitine (**9**). Elution of the column with $CHCl_3$-MeOH (95:5) gave a mixture (2.3 g) which was chromatographed on SiO_2 column. Elution with $CHCl_3$-MeOH (9:1) gave **9**[16] (312 mg) m.p. 142-143° (EtOAc); $[\alpha]_D$ 93° (*c*, 0.4 in MeOH).

Stepharine (**17**). Elution of the column with $CHCl_3$-MeOH (92:8) gave a mixture which was applied to plc on silica gel plates (solvent: $CHCl_3$-MeOH, 92:8) to give **17** (125 mg), m.p. 176-179° ($Me_2C{=}O$) (lit[20] 179-181°); $[\alpha]_D$ + 144° (*c*, 1.02 in $CHCl_3$).

Chromatography of the mixture (B) of phenolic bases. The mixture (B) of phenolic bases (35 g) was chromatographed over a column of neutral alumina (1.5 kg). The column was eluted with hexane-C_6H_6 (1:1), C_6H_6, C_6H_6-EtOAc (3:1, 1:1 and 1:3), EtOAc and EtOAc-MeOH containing an increasing proportion of the polar solvent. Elution was monitored by tlc. Fractions (320 × 200 ml) were collected. The early chromatographic fractions contained mostly already isolated non-phenolic bases. The phenolic bases isolated from the late chromatographic fractions are as follows:

Cocculine (**1**). Elution of the column with C_6H_6-EtOAc (1:3) (single spot on tlc) gave **1** (2.7 g) m.p. 216-217° (Me_2CO); $[\alpha]_D$ + 268° (*c*, 1.04 in MeOH) (lit[7] 217-218°).

Isococculine (**6**). The late fractions of C_6H_6-EtOAc (1:3) elution gave a crude base which was subjected to plc (twice) (plates: silica gel, solvent: $CHCl_3$-MeOH, 93:7) to give **6**[13] (113 mg) m.p. 182-184° $[\alpha]_D$ + 164° (*c*, 1.0 in $CHCl_3$).

Coccuvine (**7**). The mixture obtained from early fractions of EtOAc elution was rechromatographed on neutral alumina. Elution with $CHCl_3$-MeOH (98:2) yielded **7**[14] (270 mg) m.p. 137-138°.

Coccolumine (**13**). The mixture obtained from the elution of the column with C_6H_6-EtOAc (1:1) was rechromatographed on SiO_2 column. Elution with $CHCl_3$-MeOH (95:5) gave **14**[17] (110 mg) m.p. 174-175° (MeOH).

Cocculumine (**12**). The late fractions of EtOAc elution gave a mixture which by plc (plates, silica gel, solvent: $CHCl_3$-MeOH, 86:14) gave **12** (127 mg) as amorphous powder, λ_{max} 233 and 282; λ_{max} (EtOH-NaOH) 305 nm; ν_{max}: 3400 (OH), 2850, 1600, 1570, 1450, 1330, 1240 and 1095 cm^{-1}; *m/e* 301 (M^+), 286, 269 (base peak), 254, 238, 209, 198, 185, 163, 164 and 149.

O-Acetylcocculumine. A mixture of **12** (32 mg), pyridine (0.5 ml) and Ac_2O (0.5 ml) was left at room temp for 30 hr and

worked up to give O-*acetylcocculimine* (25 mg); ν_{max} 1660 and 1210 cm^{-1}; *m/e* 343 (M^+), 328, 311, 310, 296 and 280 (base peak), τ 7.8 (3 H, S, OCOMe), 6.75 (3 H, S, 3-OMe), and 6.72 (3 H, S, 11-OMe).

O-*Methylcocculine* (**2**). To a soln of **1** (69 mg) in MeOH (1 ml) was added an excess of ethereal CH_2N_2. The resulting mixture was kept at ambient temp for 60 hr and worked up in the usual way to give **2** m.p. 86–87°, identical with cocculidine (2) (m.p., mxd m.p., Co tlc, IR and NMR).

O-*Methylisococculine* (**5**). To a soln of **6** (58 mg) in MeOH (1 ml) was added an excess of ethereal CH_2N_2 to give O-*methylisococculine* (34 mg) m.p. 95–96°, identical with **5** (m.p., mxd m.p., Co-tlc, IR and NMR).

O-*Methylcoccoline* (**13**). Coccoline (**14**) (71 mg) in MeOH (1 ml) was treated with an excess of ethereal CH_2N_2 to give **13** (49 mg) m.p. 174–175, identical with coccolinine (m.p., mxd m.p., Co tlc, IR and NMR).

Dehydration of cocculitine. Cocculitine (**9**) (110 mg) in dry pyridine (2 ml) was treated with methanesulphonyl chloride (0.5 ml) in dry pyridine (1 ml) at 0° during 1 hr. The mixture was diluted with water, adjusted to pH 8 with Na_2CO_3aq and extracted with $CHCl_3$ (5 × 10 ml). The extract was washed with water, dried (Na_2SO_4) and solvent removed. The crude product, so obtained, was subjected to plc to give (in minor amount) **8** (identical with authentic sample, Co tlc).

Reduction of coccudinone. To a soln of **11** (220 mg) in MeOH (5 ml) was added $NaBH_4$ (350 mg) during 2.5 hr. The solvent from the resulting mixture was removed after 4 hr, water added, the product extracted with $CHCl_3$, washed with water, dried (Na_2SO_4) and solvent removed. The crude product, so obtained, was subjected to plc on alumina GF plates to give **9** (110 mg) m.p. 141–142° and epicocculitine (42 mg) m.p. 70–72°.

Oxidation of cocculitine. Cocculitine (**9**) (120 mg) in $CHCl_3$ (5 ml) was shaken with active MnO_2 (210 mg). The resulting mixture was worked up after 3 hr. The crude product, thus, obtained was purified on plc on silica gel plates (solvent: $CHCl_3$–MeOH, 90:10) to give **11**, (identical with authentic sample Co tlc, IR and NMR).

N-*Methylstepharine* (**18**). A mixture of **17** (110 mg), HCHO (2 ml) and HCOOH (2 ml) adjusted to pH 4–5 with Na_2CO_3 was heated on a steam bath for 1 hr. The volatile reactants were removed and the residue extracted with 2% HCl. The acidic soln was extracted with ether, basified with Na_2CO_3 and the liberated base extracted with $CHCl_3$, washed with water, dried (Na_2SO_4) and solvent removed. The crude base thus obtained was purified by plc on silica gel plates to give **18** (39 mg) m.p. 125–127° (lit[20] 127–129°).

Extraction of stems and roots. Air dried stems and roots (3 kg) of *C. laurifolius* were pulverised and percolated with EtOH (4 × 4 l). The solvent from the combined percolate was removed under reduced pressure and the viscous mass, so obtained, was extracted with 5% HCl (6 × 250 ml). The aqueous acidic soln was defatted with light petroleum ether, basified (pH 9–10) with $NaHCO_3$ and extracted with $CHCl_3$ (6 × 250 ml). Removal of the solvent from the $CHCl_3$ extract gave the alkaloidal mixture (16 g).

Chromatography of the alkaloidal mixture. The alkaloidal mixture (10 g) was chromatographed over a column of neutral alumina (400 g). The column was eluted with hexane, hexane–C_6H_6 (1:1), C_6H_6, C_6H_6–EtOAc (3:1, 1:1, 1:3), EtOAc and EtOAc–MeOH containing an increasing proportion of the polar solvent. Elution was monitored by tlc. The early fractions contained mostly the bases isolated earlier from leaves.

N-*Methylcoclaurine.* Elution of the column with EtOAc–MeOH (98:2) gave a mixture which was subjected to plc on silica gel plates (solvent: $CHCl_3$–MeOH, 85:15) to give N-*methylcoclaurine* (25 mg) m.p. 177–178° (lit[40] 178°), identical with authentic sample (m.p. mxd, mp, Co-tlc, IR, NMR and MS).

Reticuline. The mixture obtained from the middle fractions of EtOAc–MeOH (98:2) elution when applied on plc on silica gel plates (solvent: $CHCl_3$–MeOH, 88:12) gave *reticuline* (22 mg), as amorphous powder; base picrate, m.p. 189–190° (lit[41] 190–192°), identical with an authentic sample (m.p., mxd m.p., Co-tlc, IR, NMR and MS).

Coclaurine. The mixture obtained from EtOAc–MeOH, (96:4) elution when subjected to plc on silica gel plates (solvent: $CHCl_3$:MeOH, 85:15) gave *coclaurine* (78 mg), $[\alpha]_D$ + 13° (*c*, 1.02 in MeOH); base hydrochloride m.p. 245–247° (lit[42] 247–248°).

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