The Constituents of *Nauclea diderrichii*. Part III. Indole-Pyridine Alkaloids

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Six alkaloids isolated from the bark of *Nauclea diderrichii* have been shown to have structures that contain both indole and pyridine units. Because of the small amounts available of each constituent, investigation of structure has been confined mainly to the application of spectroscopic techniques; these have shown that all of these alkaloids belong to the novel indole-pyridine class, and have led us to propose structures or part structures with varying degrees of confidence for each of the alkaloids. Structure **1** was deduced for naucledine, $C_{18}H_{15}N_3O_2$, and confirmed by synthesis. Its spectroscopic characteristics indicate that naucleder ine, $C_{19}H_{19}N_3O_2$, has structure **3**, but this has not yet been confirmed by synthesis. Evidence is presented that nauclechine, $C_{21}H_{21}N_3O_3$, has part structures **6** and **7**, and it is suggested that these can be incorporated in structure **8**. A working hypothesis for the structure of nauclexine, $C_{18}H_{17}N_3O_2$, is represented by **9**. Two alkaloids, ND-363C, $C_{21}H_{21}N_3O_3$, and ND-305B, $C_{19}H_{19}N_3O$, are closely related, the latter being formally derived from the former by removal of a carbomethoxy group; each appears to exist as an equilibratible pair of isomers containing an indolic (substituted β-carboline) and a pyridine part linked together in a manner which may incorporate a spiro carbinolamine ether (as in **12**) that can form ring-opened or solvated derivatives in some solvents.

Il a été montré que six alcaloides isolés à partir de l'écorce de *Nauclea diderrichii* ont des structures comprenant à la fois des unités indole et pyridine. Par suite des petites quantités disponibles, la détermination de structure s'est faite essentiellement par des techniques spectroscopiques; celles-ci ont montré que tous ces alcaloides appartiennent à la nouvelle classe indole-pyridine. Cela nous a conduit à proposer des structures ou des parties de structure, dépendant du degré de confiance pour chacun des alcaloides. La structure **1**, proposée pour la naucl édine, $C_{18}H_{15}N_3O_2$, a été confirmée par synthèse. Les propriétés spectroscopiques de la naucl édérine, $C_{19}H_{19}N_3O_2$, nous font proposer la structure **3**, mais cela n'a pas encore été confirmé par synthèse. Des preuves sont présentées d'après lesquelles la naucléchine, $C_{21}H_{21}N_3O_3$, aurait en partie les structures **6** et **7**, et il est suggéré que celles-ci peuvent être incorporées dans la structure **8**. Une hypothèse de travail pour la structure de la naucléxine, $C_{18}H_{17}N_3O_2$, est représentée par **9**. Deux alcaloides, ND-363C, $C_{21}H_{21}N_3O_3$, et ND-305B, $C_{19}H_{19}N_3O$, sont étroitement apparentés; le dernier dérivant du premier par abstraction du groupe carbométhoxy; chacun semble exister sous forme d'une paire d'isomères équilibrable, contenant une partie indolique (β-carboline substituée) et une partie pyridinique, liées entre eux d'une façon qui pourrait faire intervenir un spiro carbinolamine éther (comme dans **12**) qui peut donner des produits d'ouverture de cycle ou des produits solvatés dans certains solvants.

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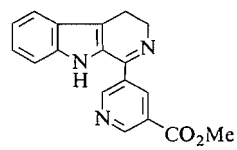
We have recently described the isolation from the bark of *Nauclea diderrichii* of a considerable number of constituents which we have placed for convenience in four categories (1, 2). The alkaloids of the first two categories, β-carbolines and pyridines, have already been described in detail (2) and we turn now to the third category, a novel group of alkaloids which we have called the indole-pyridines. Six alkaloids have been placed in this category, and although the complete structure of only one of them has been established with complete confidence at this time, their structural novelty leads us to report the evidence which allows us to place them in this category and to assign to each a provisional structure or part structure. Since the appearance of our preliminary communication (1), the isolation from another member of the Rubiaceae of two other alkaloids of this class has been reported (3).



1

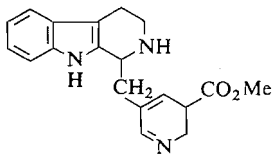
The first alkaloid, which we shall call naucledine, has been assigned structure **1**. It was isolated as crystals which melted over the range 84–90°; the wide range may result from the thermal instability of the compound, a dihydro-β-carboline. The formula, $C_{18}H_{15}N_3O_2$, was derived from its mass spectrum, which showed

a strong parent ion and, apart from a strong M − 1 peak, fragment ions of low relative intensity. The complex u.v. spectrum included a prominent peak at 328 mμ which showed a marked bathochromic and hyperchromic change in the presence of acid; this appearance and behavior is very similar to that described for the spectra of 1-aryl-3,4-dihydro-β-carbolines (4). The i.r. spectrum showed the sharp peak at 2.90 μ characteristic of an unassociated indolic NH group and carbonyl absorption near 5.80 μ which resembled that ascribed to the methyl ester functions of the pyridine alkaloids described previously (2). The characteristic signals of the aromatic protons of an indole appeared in the n.m.r. spectrum between τ 2.2 and 3.0 and the signals associated with a 3,5-disubstituted pyridine (2) appear at τ 0.81, 0.85, and 1.33; treatment of the sample with NaOD in D_2O was required to remove broadening of the pyridyl protons, and this also removed the indolic NH signal. The *O*-methyl signal was at τ 6.04, and the remaining four protons produced a symmetrical pattern of two-proton multiplets ("triplets") at τ 5.90 and 7.00 which resembled the pattern produced by the corresponding methylene groups of oxogambirtannine (5).

The structure of naucledine, **1**, was deduced from the results outlined here, and confirmed by synthesis of **1** from the acid chloride of 5-carbomethoxy-3-pyridinecarboxylic acid, available from the synthetic work we have already described in connection with the pyridine alkaloids (2). The synthesis was effected by the reaction of the acid chloride with tryptamine and the cyclization of the amide so formed to **1**, identical with natural naucledine, under Bischler–Napieralski conditions. As a preliminary to this synthesis we carried out the corresponding reactions starting with the model compounds 3- and 4-pyridinecarboxylic acid in order to discover conditions suitable for each step; this exploration was all the more necessary in the light of a report (6) that the Bischler–Napieralski cyclization failed with the amide derived from tryptamine and 3-pyridinecarboxylic acid. Our initial efforts confirmed that the cyclization failed under the conditions described (6), but we found that cyclization of the amides was successful in refluxing phosphorus oxychloride, albeit with extremely low conversion in the case of the isomer derived from 4-pyridinecarboxylic acid, and these conditions were then used in the synthesis of naucledine. In each case these Bischler–Napieralski conditions led to the formation of a by-product which appeared to be the aromatized β-carboline.

The second alkaloid in this category, which we shall refer to as naucledérine, has the formula $C_{19}H_{19}N_3O_2$ and is crystalline, but, once again, our samples melted over a considerable range, 102–124°, and showed signs of resolidifying and remelting at a higher temperature. The u.v. spectrum with a maximum at 276 mμ (ε, 9550) and a shoulder at 290 mμ could be accounted for as the summation of separate indole and pyridine chromophores (in contrast to the case of naucledine), and the i.r. spectrum showed N—H absorption, including a sharp 2.90 μ peak, and the 5.80 μ absorption characteristic of the ester group of the pyridine alkaloids described previously (2). The n.m.r. spectrum showed the patterns characteristic of aromatic protons of an indole and of a 3,5-disubstituted pyridine, and a three-proton singlet at τ 6.14 indicated that a methyl ester was present; one of the aliphatic protons formed an apparent triplet with 4 Hz spacings at τ 5.73 and the remainder were responsible for a complex pattern near τ 6.8. Peaks, which were removed when the sample was treated with D_2O, appeared at τ 1.61 and 7.55 and were attributed to the NH protons of an indole and an aliphatic secondary amine, respectively; support for the latter assignment was obtained when naucledérine formed an amide on acylation.

If it is assumed that naucledérine is derived from tryptamine without modification of the $—CH_2CH_2N<$ unit, three structures (**2**, **3**, **4**) must be considered for the alkaloid on the basis of the evidence presented so far. The mass spectrum provided evidence that structure **2** is a very unlikely possibility since it contained no significant peak at *m/e* 171, which corresponds to the most abundant fragment ion in the spectrum of 1-alkyltetrahydro-β-carbolines (7). Instead, the base peak was at *m/e* 279 ($M - C_2H_4N$ by accurate mass measurement), and the molecular ion peak was next in intensity, followed by a strong peak corresponding to $M - CH_3N$. Fragmentations of **3**



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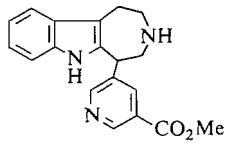

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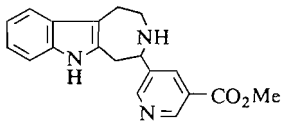

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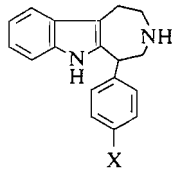

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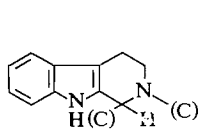
leading to the observed ions can be postulated, but it is more difficult to provide a rational explanation for the loss of C_2H_4N as the principal fragmentation of a molecule of formula **4**. Compounds of formula **5** have been described recently (8) and we are extremely grateful to Dr. K. Freter for generous samples of **5** (X = H and X = Cl). These show a marked similarity to naucleferine in their spectroscopic characteristics, particularly significant being the mass spectral fragmentation pattern which corresponded closely to that of naucleferine, especially with respect to the most intense peaks. The n.m.r. spectrum of **5** (X = Cl) also resembled that of naucleferine in showing the signal for one proton as an apparent triplet with 4.5 Hz spacings at τ 5.79, a multiplet near τ 6.8 attributed to the aliphatic protons, and the signal for the NH proton of the seven-membered ring at τ 7.63. The evidence presented here leads us to propose with a considerable degree of confidence that naucleferine has structure **3**, and we are currently attempting to establish this with complete certainty by a total synthesis.

The third alkaloid of this class, given the name nauclechine, m.p. 108–114°, has the formula $C_{21}H_{21}N_3O_3$. As with naucleferine, its u.v. spectrum is compatible with the presence in the molecule of separate indole and pyridine chromophores, and its i.r. spectrum shows strong absorption in the 2.8–3.1 μ region with a sharp peak, appropriate for an indolic NH, at 2.91 μ, and a carbonyl peak at a wavelength, 5.83 μ, very slightly longer than that observed previously in the spectra of the esters of 3-pyridinecarboxylic acids (2). A three-proton singlet at τ 5.94 in the n.m.r. spectrum supported the proposal that we again had the methyl ester of a pyridinecarboxylic acid, and the signals for the aromatic protons and the exchangeable NH proton of an indole were also present; when the sample was treated with D_2O, in addition to the loss of the indole NH signal at τ 1.28, a peak was lost from the envelope near τ 6.2, and this peak is attributed to the OH proton of an alcohol. The n.m.r. spectrum also provided evidence that the molecule contained a pyridine unit, but, in this case the signals observed were one-proton singlets at τ 0.98 and 1.42, and are assigned to the protons at positions 2 and 6 of a 3,4,5-trisubstituted pyridine. The part of the spectrum associated with aliphatic protons is complex, but a one-proton doublet, $J = 6.5$ Hz (slightly broadened by further weak coupling) at τ 5.08 is well separated on the low field side, and is attributed to a proton on a carbon atom carrying an OH group and attached to a pyridine ring; double irradiation showed that this proton was coupled to a proton at τ 6.49, which appeared to have an additional coupling, $J = 12.5$ Hz, to a third proton at τ 7.19; two slightly broadened peaks at the edge of the aliphatic envelope can be accounted for as the low field component (a doublet at τ 5.80, $J = 15.0$ Hz) of a second, independent three-spin system in which the proton at lowest field is coupled strongly ($J = 15.0$ Hz) to a proton at τ 6.54 and weakly ($J < 1$ Hz) to a proton at τ 6.22, and the latter two protons are themselves coupled with $J = 9.0$ Hz. In the mass spectrum the molecular ion at *m/e* 363 provides the base peak, an intense M − 1 peak is present, and strong peaks are observed at *m/e* 184, 183, 170, 169, and 156, a pattern that is characteristic of the tetrahydro-β-carbolines, such as yohimbine, in which positions 1 and 2 are further annelated; the spectrum in these regions bears, in fact, a strong resemblance to that of ajmalicine, and accurate mass measurement of the ions listed shows, furthermore, that they have the same formulae as those appearing in the fragmentation of ajmalicine (9). In addition, the spectrum has peaks at *m/e* 332 (M − OCH_3) and 346 (M − OH), and these fragmentations, verified by accurate mass measurements, are characteristic of the methyl ester of an aromatic carboxylic acid and a benzylic-type alcohol, respectively (10).

The evidence presented here is entirely in accord with the presence of both of the part



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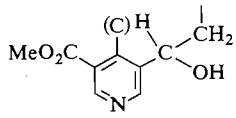

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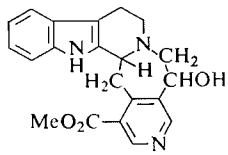
structures **6** and **7** in nauclechine. In particular, the mass spectral evidence for **6**, with no further substitution of the tetrahydro-β-carboline, is compelling; the n.m.r. evidence requires a 3,4,5-trisubstituted pyridine as in **7** and the n.m.r., i.r., and mass spectra all indicate the types of substituents illustrated, but they do not show unequivocally that the groups are in the order shown. The order appears probable when comparison is made with the simple pyridine alkaloids described previously, and when **6** and **7** are combined in **8**, a credible explanation can be given of all of the available data. In addition to the features already dis-



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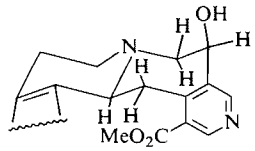


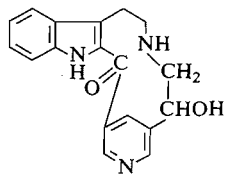
8*a*

cussed with respect to the individual part structures, the protons on the seven-membered ring incorporated in **8** would be expected to give rise in the n.m.r. spectrum to two separate three-spin systems, and the analysis outlined above shows that the spectrum does satisfy this requirement. A reasonable stereochemical arrangement (**8***a*) of the molecule can be suggested in explanation of the coupling constants observed: in each three-spin system the methylene protons have a strong mutual coupling, but only one proton in each pair has the appropriate angular relationship with the vicinal proton to couple appreciably with it; the chemical shift assignments are reasonable, with the proton on the carbon bearing the OH group being at lowest field (τ 5.08) in one set, but in the other three-spin set, the low field resonance (τ 5.80) must be assigned to the quasi-equatorial methylene proton, and its unusually low value must be associated with the location of the proton in the plane of the pyridine ring and adjacent to the carbomethoxy group. The presence of a Bohlmann band (11) in the i.r. spectrum is in accord with the suggested stereochemistry.

In summary, we propose that the structure of nauclechine is constituted of the components **6** and **7**; **8** represents a combination of these part structures that is entirely in accord with the available data, but the evidence falls short of establishing unequivocally that nauclechine has this structure.

The fourth alkaloid, which we shall refer to as nauclexine, was isolated as a small amount of crystalline substance, $C_{18}H_{17}N_3O_2$, m.p. 229–232°, from only one lot of bark. Its u.v. spectrum, which clearly distinguishes it from the other alkaloids we have obtained, indicates that it is a 2-acylindole, but its intense absorption at 300 mμ, which is unaffected by the addition of either acid or base, is at a wavelength 10–15 mμ shorter than that of typical 2-acylindole alkaloids (12). Its i.r. spectrum shows a very strong peak at 6.13 μ at the upper end of the range associated with the carbonyl group of 2-acylindole alkaloids and about 0.05 μ above a typical value (12); the i.r. spectrum also shows a sharp peak at 2.91 μ, indicative of an indolic NH, superimposed on a broader peak centered at about 3.05 μ. Because of the poor solubility of nauclexine in chloroform-*d*, its n.m.r. spectrum was obtained under special conditions (see Experimental). In the aromatic region of the spectrum, evidence of an indole was present, but the broadening of the lower field peaks associated with protons on a pyridine ring did not allow a confident assignment of the substitution pattern on the pyridine ring to be made. The only other useful deduction that can be drawn from the spectrum is that a multiplet at τ 4.89 has the characteristics expected for the signal of a proton on a carbon attached to a pyridine ring and carrying an OH group. There is, therefore, presumptive evidence that nauclexine contains a disubstituted pyridine ring and a 2-acylindole unit. If the molecule retains the $—CH_2CH_2N<$ unit of a tryptamine precursor, only one carbon atom remains unassigned and this must be placed between the nitrogen and the CHOH unit since the alkaloid does not appear to be a carbinolamine.

If all of these deductions were firmly grounded, and the usual 3,5-disubstitution pattern of the pyridine ring could be established, structure



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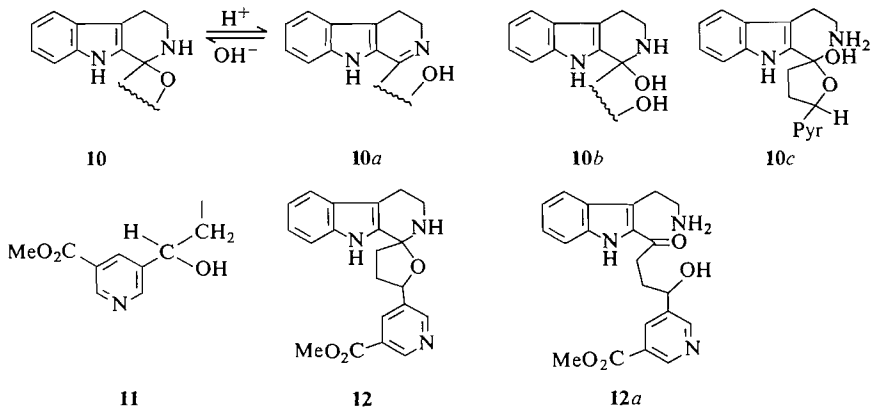
9 would represent nauclexine; unfortunately the quality of the data does not permit us to go further than suggesting this interesting structure as a working hypothesis. The unusual 11-membered ring, produced by a meta-bridging of the pyridine ring, has the virtue of providing an explanation for the failure of the amine and ketone to form a transannular carbinolamine bridge since such a bridge would produce a very highly strained system; however, a severe constraint of this sort is not obligatory since the position of the equilibrium between the amino ketone and carbinolamine forms can depend on more subtle stereochemical features than this (13). The mass spectrum of nauclexine is compatible with structure **9**. It is characterized by the presence of an extremely intense peak at *m/e* 199 ($C_{12}H_{11}N_2O$ by accurate mass measurement), much stronger than any other peak in the spectrum, which can be assigned to an ion resulting from loss of the pyridine by fragmentation at the picolyl position and, accompanied by a hydrogen transfer, cleavage between the carbonyl group and the pyridine ring. Peaks corresponding to those in picraphylline and burnamicine (14) were observed at *m/e* 129, 130, 143, and 144; significant peaks were also observed at *m/e* 157, 169, 170, 171, and 184. The absence of a peak corresponding to M − OH was unexpected, but it may be noted that all of the peaks corresponding to ions, including the parent ion, of higher mass than the base peak were of low intensity.

The next alkaloid, which we shall refer to by its laboratory designation ND-363C (the number indicating the assigned molecular weight), was isolated as a viscous syrup which, on the basis of its t.l.c. behavior, appeared to be homogeneous. The formula $C_{21}H_{21}N_3O_3$ was assigned to it by accurate mass measurement of the parent ion in the mass spectrum, and evidence was seen in the i.r. spectrum that we once again had a molecule which was both an indole (2.90 μ) and a carbomethoxypyridine (5.80 μ). The u.v. spectrum was extremely distinctive: ND-363C, in methanol containing added alkali, produced a spectrum with absorption of moderate intensity between 250 and 300 mμ that was very similar to that of the alkaloids containing separate indole and pyridine chromophores, while a solution in methanol containing added acid produced a dramatically different spectrum, characterized by intense absorption at 364 mμ, and the spectrum of a solution in methanol alone showed both sets of peaks with intensities between the limits in acid and alkali. The n.m.r. spectrum of ND-363C is extremely complex, but a considerable simplification in its interpretation can be achieved if it is postulated that it arises from two closely related species present in equal concentrations so that each set of peaks is doubled (but sometimes coincident). In the low field region of the spectrum, four doublets, at τ 0.85, 0.99, 1.17, and 1.50, and two triplets, at τ 1.68 and 2.20, can be assigned to overlapping patterns resulting from two 3,5-disubstituted pyridines; complex absorption between τ 2.5 and 3.0 has an integrated area on this basis corresponding to the aromatic protons of two indole moieties; multiplets at τ 4.70 and 5.08 each of which has the appearance of an X part of an AMX system, can each be assigned to one proton and singlets at τ 6.09 and 6.34 are associated with two different methyl esters; signals that correspond to protons exchangeable with D_2O are present at τ 0.99 and 8.22 but it is difficult to be confident about the number of protons related to them, the low field peak being reasonably assigned on the present basis to the superimposition of the NH signals of two indole units but the high field peak being considerably more intense (it could correspond to as many as three protons on each of the two molecular species); the remaining signals fall between τ 6 and 7.5 and are complex and poorly resolved.

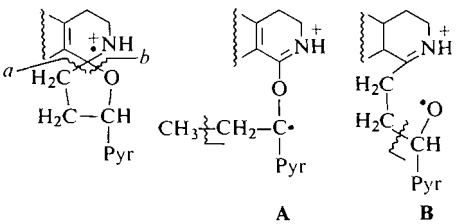
The doubling of the n.m.r. spectrum could arise if ND-363C were a dimeric or pseudodimeric molecule (15), but we have no evidence that the molecular weight is higher than the assigned value, and at present we prefer to explain our results by proposing that ND-363C exists as two isomers that can be readily equilibrated and are, therefore, inseparable by t.l.c. The u.v. spectra of ND-363C are compatible with the presence in the molecule of the car-



binolamine ether of part structure **10** which can equilibrate with the open form **10***a*; in addition, the i.r. and n.m.r. evidence points to the presence of two units of part structure **11**, the multiplets at τ 4.70 and 5.08 being taken as evidence for the presence of a hydrogen on a carbon bearing an oxygen, a methylene group and a pyridine ring. If **10** and **11** are now combined in **12**, it can be seen that ND-363C could well exist in chloroform solution as the two diastereoisomers of **12**, epimeric at the spiro junction, equilibratible through **10***a*, of comparable free energy, and therefore present in approximately equal concentrations. Evidence contrary to this hypothesis is seen in the u.v. spectrum of ND-363C in neutral methanol where it is assumed that a considerable amount of the form **10***a* is present and it is necessary to argue that the discrepancy arises because of the different solvents used to obtain the u.v. and n.m.r. spectra. It is more difficult to find a satisfactory explanation for the presence at τ 8.22 in the n.m.r. spectrum of strong peak that is removed by treatment of the sample with deuterium oxide. It is tempting to suggest that the equilibrium mixture contains a further component that is a hydrated species such as **10***b* or *c*, and it can be seen that all of these structures can be derived, at least formally, by cyclizations of the open keto form, **12***a*, of the molecule through condensations of the carbonyl group with the hydroxy or amino functions or both; it should be noted, however, that only those derivatives which are chiral at both carbon centers bearing an oxygen function can exist as diastereoisomeric pairs and that the n.m.r. evidence can be satisfied only if all of the protons on oxygen or nitrogen have effectively the same chemical shift, but interconversion of diastereoisomers is not rapid enough to produce time-averaged signals in the rest of the molecule. The mass spectrum provides no evidence that the isolated sample is hydrated, and, although it is possible that a complex equilibrium between different forms, each of which predominates under different conditions, may supply the complete explanation, further work, when we have isolated sufficient alkaloid, will be required to establish this.

The mass spectrum of ND-363C provided evidence that the molecular formula is $C_{21}H_{21}N_3O_3$ and that no ions of higher mass, corresponding to a dimeric alkaloid and its fragment ions or a hydrated species, could be found; the appearance of peaks at *m/e* 130, 156, and 169 and the absence of an appreciable M − 1 peak supported the postulate that the structure was based on a tetrahydro-β-carboline which lacked a hydrogen at C-1 (9), and the presence of a peak corresponding to M − OCH_3 was in accord with the presence of the methyl ester of a pyridinecarboxylic acid (10). However, two peaks in the spectrum present problems on first examination; the base peak at *m/e* 348, which corresponds to M − CH_3 and the prominent peak at *m/e* 198. The loss of a methyl radical is a rare but not unprecedented fragmentation process (16) if the molecule does not have a preformed methyl group but we know of no case in which such a fragmentation produces the base peak. The n.m.r. spectrum of ND-363C provided no evidence for the presence of a methyl group, other than that of the ester function which would not be expected to frag-

ment by loss of a methyl radical (10). We have sought to reconcile these conflicting pieces of evidence by utilizing a distinctive feature of **12**. An important site of cleavage of the molecular ion of **12** can be expected to be the spiro center, and two ions formed by rupture at *a* or *b* are of particular significance, the former leading, after transfer of a hydrogen, to ion **A** which can be expected to form a stable fragment



A **B**

ion by loss of a methyl radical; rupture at *b* would lead to ion **B** which would then lose the pyridyl portion of the molecule along with the CHO unit as an uncharged fragment and leave an ion of *m/e* 198.

A further alkaloid, designated ND-305B, appears to be very closely related to ND-363C, and, indeed, all of the observable properties of ND-305B can be accounted for on the basis that its structure is the same as that of ND-363C with the carbomethoxy group removed. It has the formula $C_{19}H_{19}N_3O$, its i.r. spectrum lacks the carbonyl absorption but retains the NH absorption, and its u.v. spectrum resembles that of ND-363C both in appearance and in behavior in acid or base. The n.m.r. spectrum of ND-305B shows a very close correspondence to that of ND-363C except that the methoxyl signals are now absent and the protons of the pyridine ring lead to a complex multiplet in the low field region of the spectrum. As with ND-363C, the problem regarding the presence of a high field signal, now at τ 8.21 due to protons exchangeable with D_2O also remains, as does the question of the origin of peaks at $M - CH_3$ and *m/e* 198 in the mass spectrum of ND-305B; the latter data do, however, demonstrate that the methyl radical is not lost from a carbomethoxy group (in ND-363C such a process was conceivable but highly improbable), and that the *m/e* 198 peak arises from the portion of the molecule that does not contain the pyridine ring. In short, the above discussion of the nature of ND-363C applies equally well to ND-305B, except that the carbomethoxy group is absent in the latter, and the results obtained for ND-305B buttress to some extent the conclusions based on ND-363C alone. The alkaloids, apparently equilibratible pairs of isomers, contain an indole (substituted β-carboline) part and a pyridine part which are linked together in a manner that has not yet been fully explained, but probably involves a function such as a carbinolamine ether that allows equilibration through a reversible cyclization.

In summary, the evidence presented allows us to place the six alkaloids in the novel class we have called the indole-pyridines; the complete structure of naucledine has been deduced and confirmed by synthesis, while for the other alkaloids additional features of their structures have been described and structural proposals have been advanced with varying degrees of confidence. However, there still remains the question of whether or not these are truly natural products, or whether they are artifacts produced in isolation procedures which used ammonia (2). It is known that some so-called pyridine alkaloids arise in this way (17). In our studies we have attempted to rule out this possibility by carrying out an isolation and separation of the alkaloids by a procedure that rigorously avoided the use of ammonia (2). Unfortunately, the only base isolated and identified on that occasion was a simple pyridine (compound **6** in ref. 2); while this result shows that the pyridine nucleus did not necessarily arise by the incorporation of ammonia used in the isolation procedure, the absence of the more interesting and more complex indole-pyridines described above is cause for concern. However, if these substances were artifacts, they would nevertheless have been the transformation products of natural precursors which were, themselves, structurally related indole alkaloids, and these were not isolated either under these conditions. We have noted previously (2) that the yields of individual alkaloids from different batches of bark were extremely variable and we consequently consider that the status of the indole-pyridines as natural products is still not proven; their interest as chemical entities remains, regardless of their origin.

Experimental

A description of routine instrumentation, spectroscopic conditions, extraction procedures, etc. has already been given (2).

Naucledérine

Naucledine was separated in t.l.c. systems of relatively low polarity. It was recrystallized from methanol and obtained as pale yellow needles, melting over the range 84–90°. Spectroscopic characteristics: i.r. 2.90 (sharp), 3.07 (broad), 5.79, 5.82 (shoulder); u.v. 220 (26 000, end absorption), 232 (shoulder), 248 (shoulder), 267 (shoulder), 328 (8900); 352 (shoulder); in MeOH–HCl 220 (18 000; end absorption), 252 (9800), 269 (shoulder), 306 (13 000); n.m.r. (solution treated with 6% NaOD in D_2O) 0.81 (d, $J = 2$ Hz; 1), 0.85 (d, $J = 2$ Hz; 1), 1.33 (3 peaks, 2 Hz spacings; 1) (prior to treatment of the sample with NaOD, these three signals gave unresolved single peaks and an additional one-proton peak appeared at 1.05), 2.2–3.0 (complex; 4), 5.90 and 7.00 (each 3 peaks, 8 Hz spacings; each 2), 6.04 (s; 3); mass spectrum 306 (20), 305 (100), 304 (85), 303 (15), 290 (3), 277 (5), 276 (4), 247 (7), 246 (8), 245 (12), 244 (11), 219 (9), 218 (18), 217 (14), 216 (6), 191 (5), 190 (7), 143 (12), 140 (6), 123 (6), 122.5 (8), 115 (8), 109 (6), 95.5 (7); metastable 303.7 (calcd. for 305 → 304: 303.1); mass measurements calcd. (found): $C_{18}H_{15}N_3O_2$, 305.11643 (305.1181); $C_{17}H_{13}N_2O_2$, 277.09770 (277.0966); $C_{16}H_{11}N_3$, 245.09530 (245.0950); $C_{15}H_{10}N_2$, 218.08440 (218.0835); $C_{10}H_9N$, 143.07350 (143.0743).

Naucledérine

Naucledérine was obtained from fairly polar t.l.c. systems. It was obtained as a syrup which was soluble in methanol and this solution deposited crystals which were then almost insoluble in methanol. The crystals melted over a wide range, typically 102–124°, partially resolidified on further heating and remelted at higher temperatures, typically in the range 172–183°. Two different samples of the crystals had $[\alpha]_D^{26}$ −13° (c 0.8, $CHCl_3$) and $[\alpha]_D^{25}$ 0° (c 3.3, $CHCl_3$). Spectroscopic characteristics: i.r. 2.90 (sharp), 3.03 (broad), 5.80, 5.82 (shoulder); u.v. 224 (40 700), 276 (9550), 290 (shoulder); n.m.r. (solution treated with K_2CO_3/D_2O) 1.03 (d, $J = 2$ Hz; 1), 1.48 (d, $J = 2$ Hz; 1), 1.88 (3 peaks, 2 Hz spacings; 1) (prior to treatment of the sample with K_2CO_3/D_2O, these three signals gave unresolved single peaks and an additional one-proton peak appeared at 1.61), 2.4–3.0 (complex; 4), 5.73 (3 peaks, 4 Hz separations; 1), 6.14 (s; 3), 6.5–7.0 (complex; 6), (2.45 (broad; 1; present only prior to treatment of sample with D_2O)); mass spectrum (a peak at 335 of variable intensity was probably produced by a contaminant), 322 (13), 321 (59), 293 (9), 292 (44), 291 (17), 280 (20), 279 (100), 277 (14), 266 (3), 247 (6), 233 (7), 219 (12), 218 (8), 204 (7), 191 (4), 167 (6), 165 (5), 156 (4), 130 (7), 57 (7), 43 (10), 42 (12); metastables 290.2 (calcd. for 292 → 291: 290.0), 265.8 (calcd. for 321 → 292: 265.7), 262.9 (calcd. for 292 → 277: 262.4), 242.5 (calcd. for 321 → 279: 242.5); mass measurements calcd. (found): $C_{19}H_{19}N_3O_2$, 321.14773 (321.1468); $C_{18}H_{16}N_2O_2$, 292.12118 (292.1210); $C_{17}H_{15}N_2O_2$, 279.11335 (279.1124); $C_{17}H_{13}N_2O_2$, 277.09770 (277.0977); $C_{16}H_{14}N_2O_2$, 266.10553 (266.1051); $C_{15}H_{11}N_2$, 219.09222 (219.0914); $C_{10}H_{13}N_3O$, 191.10586 (191.1057); $C_8H_{13}N_3O$, 167.10586 (167.1056). *p*-Bromobenzoyl chloride in pyridine reacted with the alkaloid to form a crystalline derivative, m.p. 207–210°; i.r. 2.90 (sharp), 3.06 (broad), 5.80, 5.83 (shoulder), 6.15; mass measurements calcd. (found): $C_{26}H_{22}N_3O_3{}^{81}Br$, 505.08254 (505.0828); $C_{26}H_{22}N_3O_3{}^{79}Br$, 503.08451 (503.0835); $C_{19}H_{16}N_2O_2$ (fragment ion) 304.12118 (304.1212).

The model 5-phenylhexahydroazepinoindole **5** (X = H) kindly provided by Dr. K. Freter (Pharma-Research Canada, Ltd.) provided the following mass spectrum: 263 (10), 262 (47), 234 (5), 233 (27), 232 (16), 230 (4), 221 (18), 220 (100), 219 (7), 218 (15), 217 (16), 204 (5), 115 (5), 108.5 (4), 43 (4), 42 (5); metastables 216.5 (calcd. for 220 → 218: 216.1), 208.1 (calcd. for 233 → 220: 207.7), 207.1 (calcd. for 262 → 233: 207.2), 203.8 (calcd. for 233 → 218: 204.0), 184.8 (calcd. for 262 → 220: 184.7); mass measurements calcd. (found): $C_{18}H_{18}N_2$, 262.14700 (262.1471); $C_{17}H_{15}N$, 233.12045 (233.1194); $C_{16}H_{14}N$, 220.11262 (220.1132). The corresponding chloro derivative **5** (X = Cl) showed: 289 (19), 297 (12), 296 (58), 294 (5), 269 (11), 268 (12), 267 (32), 266 (18), 257 (6), 256 (33), 255 (20), 254 (100), 252 (7), 232 (25), 231 (10), 230 (10), 219 (13), 218 (32), 217 (35), 216 (6), 204 (6), 148 (7), 130 (11), 115 (7), 108.5 (5), 102 (5), 43 (11), 42 (14).

Nauclechine

Nauclechine was obtained from t.l.c. systems of intermediate polarity. It was recrystallized from chilled methanol and isolated as white crystals, m.p. 108–114°. Spectroscopic characteristics: i.r. 2.91 (sharp), 3.00 (broad), 3.60 (Bohlmann band (11)), 5.83; u.v. 224 (40 700), 272 (10 050), 288 (shoulder); n.m.r. (solution treated with K_2CO_3/D_2O) 0.98 (s; 1), 1.42 (s; 1) (prior to treatment of the sample with K_2CO_3/D_2O these two peaks were broadened and an additional one-proton peak appeared at 1.28), 2.4–3.0 (complex; 4), 5.08 (2 peaks, 6.5 Hz spacing; collapsed to singlet by irradiation at 6.49; 1), 5.80 (2 peaks, 15.0 Hz spacing; 1), 5.94 (s; 3), 6–7.5 (complex; 8; included in this was a set of peaks centered at 6.49 indicating one proton with J values 12.5 and 6.5 Hz, the latter coupling being removed by irradiation at 5.08; the signals overlapped a set of peaks indicating one proton with J values 15.0 and 9.0 Hz; pairs of peaks with spacings of 9.0 and 12.5 Hz respectively appeared at 6.22 and 7.19; an additional broad signal at ≃6.2 was present only prior to treatment of the sample with D_2O); mass spectrum 364 (23), 363 (100), 362 (87), 361 (5), 348 (5), 347 (6), 346 (22), 332 (10), 303 (5), 185 (10), 184 (48), 183 (43), 182 (7), 171 (13), 170 (39), 169 (55), 168 (10), 167 (6), 157 (9), 156 (32), 155 (10), 154 (11), 144 (16), 143 (16), 142 (6), 130 (11), 129 (9), 128 (8), 127 (5), 117 (5), 116 (5), 115 (10), 86 (6), 84 (9), 77 (7), 65 (5), 57 (5), 51 (5), 49 (12), 43 (8), 42 (9), 41 (5); metastable 361.6 (calcd. for 363 → 362: 361.0); 15 eV spectrum 363 (100), 362 (29), 184 (6), 183 (6), 170 (6); mass measurements calcd. (found): $C_{21}H_{21}N_3O_3$, 363.15829 (363.1575); $C_{21}H_{20}N_3O_2$, 346.15555 (346.1551); $C_{20}H_{18}N_3O_2$, 332.13990 (332.1398), $C_{12}H_{12}N_2$, 184.10005 (184.1006); $C_{12}H_{11}N_2$, 183.09222 (183.0923), $C_{11}H_9N_2$, 169.0760 (169.07657), $C_{11}H_{10}N$, 156.0815 (156.08132).

Nauclexine

Nauclexine was obtained from t.l.c. systems of intermediate polarity. It was recrystallized from a mixture

of methylene chloride and methanol and isolated as off-white needles, m.p. 229–232°, which appeared to be weakly laevorotatory in methanol but an accurate value for the specific rotation could not be measured because of the very small amount of material available. Spectroscopic characteristics: i.r. 2.91 (sharp), 3.05 (broad), 6.13 (v. strong); u.v. 226 (17 800), 235 (14 800), 300 (15 850); n.m.r. (dilute solution in 98:2 $CDCl_3$–CD_3OD; 150 scans CAT; peak areas obtained only very approximately) 0.21 (broad; 1), 2.0–3.0 (complex; ?), 4.89 (m; 1), 6.0–7.1 (complex; 6); (dilute solution in 1:1 $CDCl_3$–CD_3OD; 150 scans CAT) additional (one-proton ?) peaks appear at 0.97, 1.36, and 1.53; the 4.89 multiplet appears to have 4 peaks with 5.0, 2.5, and 5.0 Hz spacings, and at 7.03 there is an apparent two-proton triplet, $J = 7.0$ Hz; mass spectrum 307 (9), 200 (20), 199 (100), 184 (2), 171 (5), 170 (5), 169 (8), 157 (4), 144 (10), 143 (8), 130 (6), 129 (9), 128 (5), 115 (6), 102 (4), metastables 168.7 (calcd. for 171 → 170: 169.0), 147.0 (calcd. for 199 → 171: 146.9), 145.2 (calcd. for 199 → 170: 145.2), 129.0 (calcd. for 307 → 199: 129.0), 121.3 (calcd. for 171 → 144: 121.3); 15 eV spectrum 307 (100), 199 (40); mass measurements calcd. (found): $C_{18}H_{17}N_3O_2$, 307.13208 (307.1336); $C_{12}H_{11}N_2O$, 199.08714 (199.0869); $C_{11}H_9N_2$, 169.07657 (169.0764); $C_{10}H_{10}N$, 144.08132 (144.0816); C_9H_7, 115.05478 (115.0546).

Alkaloid ND-363C

This alkaloid was separated by moderately polar t.l.c. systems and was obtained as a syrup which appeared homogeneous in five t.l.c. systems. Spectroscopic characteristics: i.r. 2.90 (sharp), 3.04 (broad), 5.80, 5.82 (shoulder); u.v. 223 (31 500), 252 (6900), 271 (7100), 291 (4800), 360 (6500); in MeOH–HCl 223 (16 200, end absorption), 250 (11 200), 266 (shoulder), 364 (18 600); in MeOH–KOH 223 (38 900), 274 (8700), 281 (shoulder), 290 (shoulder); n.m.r. (solution treated with K_2CO_3/D_2O; the number of protons assigned to each signal is based on the assumption that two $C_{21}H_{21}N_3O_3$ units are present) 0.85 (d, $J = 2$ Hz; 1), 0.99 (d, $J = 2$ Hz; 1), 1.17 (d, $J = 2$ Hz; 1), 1.50 (d, $J = 2$ Hz; 1); 1.68 (3 peaks, 2 Hz spacings; 1), 2.20 (3 peaks; 2 Hz spacings; 1), (prior to treatment of the sample with K_2CO_3/D_2O, these six signals appeared as unresolved peaks and the 0.99 signal corresponded to a total of 3 protons), 2.4–3.0 (complex; 8), 4.70 (4 peaks, spacings 6, 3, and 6 Hz; 1), 5.08 (4 peaks, spacings 6, 1, and 6 Hz; 1), 6.09 (s; 3), 6.34 (s; 3), 6.4–7.5 (complex; ≃16), 8.22 (s; ≃6; present only prior to treatment of the sample with D_2O); mass spectrum 364 (6), 363 (18), 349 (24), 348 (100), 332 (2), 316 (4), 199 (7), 198 (25), 197 (6), 172 (9), 170 (7), 169 (12), 165 (5), 164 (13), 157 (6), 156 (6), 155 (9), 154 (7), 144 (10), 143 (6), 134 (5), 130 (11), 129 (9), 128 (5), 115 (6), 104 (5), 77 (7); metastables 334.0 (calcd. for 363 → 348: 333.6), 287.0 (calcd. for 348 → 316: 286.9); 13 eV spectrum 363 (100), 348 (39), 198 (91); mass measurements calcd. (found): $C_{21}H_{21}N_3O_3$, 363.15829 (363.1578); $C_{20}H_{18}N_3O_3$, 348.13482 (348.1345); $C_{20}H_{18}N_3O_2$, 332.13990 (332.1401); $C_{19}H_{14}N_3O_2$, 316.10860 (316.1089).

Alkaloid ND-305B

This alkaloid was separated by more polar t.l.c. systems and was obtained as a syrup that appeared by t.l.c. to be homogeneous. Spectroscopic characteristics: i.r. 2.90 (sharp), 3.10 (broad); u.v. 224 (30 900), 250 (shoulder), 262 (shoulder), 284 (7800), 292 (7250), 310 (shoulder), 360 (5400); in MeOH–HCl 224 (16 200, end absorption), 251 (13 800), 365 (17 400); in MeOH–KOH 215 (47 900, end absorption), 223 (shoulder), 269 (10 250), 283 (9800), 291 (shoulder); n.m.r. (solution treated with K_2CO_3/D_2O; the number of protons assigned to each signal is based on the assumption that two $C_{19}H_{19}N_3O$ units are present) 1.3–1.5 (complex; ≃6; prior to treatment of the sample with D_2O the peak at 1.32 was higher), 2.2 (two peaks, 7 Hz spacing and fine splitting; 1), 2.47 (m; ≃1), 2.5–3.0 (complex; ≃8), 4.71 (4 peaks, spacings 6, 3, and 6 Hz; 1), 5.08 (4 peaks, spacings 6, 1, and 6 Hz; 1), 6.1–7.6 (complex; ≃18), 8.21 (s; ≃6; present only prior to treatment of the sample with D_2O); mass spectrum 305 (16), 291 (23), 290 (100), 199 (13), 198 (20), 183 (9), 182 (10), 156 (7), 155 (9), 154 (8), 144 (10), 143 (7), 130 (12), 129 (10), 128 (7), 115 (7), 106 (19), 78 (12), 77 (10); metastable 275.9 (calcd. for 305 → 290: 275.7); mass measurements calcd. (found): $C_{19}H_{19}N_3O$, 305.15281 (305.1531); $C_{18}H_{16}N_3O$, 290.12934 (290.1299).

Synthesis of 1-(3'-Pyridyl)-3,4-dihydro-β-carboline (Model Compound)

N-Nicotinoyl-β-(3-indolyl)ethylamine (125 mg; 0.47 mmol) prepared from tryptamine and the hydrochloride of nicotinoyl chloride (6), was suspended in phosphorus oxychloride (13.4 g; 87 mmol) and the mixture was refluxed for 2 h. Phosphorus oxychloride was removed on a rotary evaporator, the brown oily residue was dissolved in dilute acetic acid and, after filtration, the solution was made basic with ammonia and extracted with chloroform. The extract afforded a brown oil which was purified by preparative t.l.c. on silica gel with elution by 99:1 acetone–methanol. The product was obtained as an oil (34 mg; 28% yield) which could not be crystallized. Spectroscopic characteristics: i.r. 2.89, 6.18, 6.29, 6.37, 6.54; u.v. 213 (end absorption), 230 (shoulder), 244 (shoulder), 328 (9500); n.m.r. (solution treated with $NaOD/D_2O$) −0.85 (s; 1; observed only prior to treatment with D_2O), 1.03 (d, $J = 2$ Hz; 1); 1.68 (dd, J values 6, 2 Hz; 1), 1.96 (dt, J values 8, 2 Hz; 1), 2.42 (m; 1), 2.80 (complex; 4), 6.99 (3 peaks, 8 Hz spacings; 2), 7.06 (3 peaks, 8 Hz spacings; 2).

Synthesis of Naucledine (1)

3-Carbomethoxy-5-chlorocarbonylpyridine (2.4 g; 12 mmol) in 120 ml of carefully dried ethanol-free chloroform (2) was added to tryptamine (2.4 g; 15 mmol) dissolved in 60 ml of dry ethanol-free chloroform and 90 ml of dry pyridine, and refluxed for 2.5 h. Precipitated salts were removed by filtration and the filtrate was evaporated to dryness in a rotary evaporator. The residue was treated with 200 ml of 5% ammonia and extracted with four 100 ml portions of chloroform. The extract afforded a white residue (2.5 g) from which the tryptamide (1.0 g; 50% yield) was obtained as crystals, m.p. 168.5–169.5°, by recrystallization from methanol.

Anal. Calcd. for $C_{18}H_{17}N_3O_3$: C, 66.86; H, 5.30; N, 13.00. Found: C, 67.12; H, 5.33; N, 12.96.

Spectroscopic characteristics; i.r. (Nujol) 2.98 (sharp), 3.01 (broad), 5.85, 6.02, 6.24, 6.45; u.v. 222 (end absorption), 273 (10 100), 290 (8300); n.m.r. (DMSO-d_6) −0.90 (s; 1), 0.65 (d, $J = 2$ Hz; 1), 0.73 (d, $J = 2$ Hz; 1), 0.88 (t, $J = 5$ Hz; 1), 1.22 (3 peaks, 2 Hz spacings; 1), 2.2–3.1 (complex;

5), 6.08 (s; 3), 6.36 (m; 2), 6.96 (3 peaks, 7 Hz spacings; 2); mass spectrum 323 (10), 164 (6), 144 (18), 143 (100), 131 (13), 130 (82), 103 (10), 44 (22), 43 (51).

Treatment of the tryptamide (1.0 g; 3.1 mmol) with phosphorus oxychloride (100 g) in the manner described above for the model compound yielded a crude product (0.61 g) from which naucledine (52% yield) could be obtained by preparative t.l.c. on silica gel with elution by 99:1 acetone–methanol. The synthetic material, m.p. 84–90° (dec.) did not depress the melting point of natural naucledine and the two materials were identical in t.l.c. and spectroscopic characteristics.

Anal. Calcd. for $C_{18}H_{15}N_3O_2$: C, 70.80; H, 4.95; N, 13.76. Found: C, 70.66; H, 5.00; N, 13.50.

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