The Constituents of *Nauclea diderrichii*. Part II. Isolation and Classification of Constituents; Simple β-Carboline and Pyridine Alkaloids[1]

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The bark of *Nauclea diderrichii* (Rubiaceae) has afforded a considerable number of constituents, mainly alkaloids. For convenience these have been placed in four categories: (*1*) simple β-carbolines, (*2*) simple pyridines, (*3*) indole-pyridines, and (*4*) miscellaneous substances. Isolation procedures and the elucidation of the structures of constituents in categories (*1*) and (*2*) are described in this paper. Spectroscopic evidence supported by synthesis has been used to assign structures **1**, **2**, **3**, and **4** to the four simple β-carbolines and structures **5**, **6**, **7**, and **8** to the four simple pyridines isolated.

L'écorce du *Nauclea diderrichii* (Rubiaceae) a donné un nombre considérable de produits constitués surtout d'alcaloides. Dans un but pratique, ceux-ci ont été classés en quatre catégories: (*1*) les β-carbolines simples, (*2*) les pyridines simples, (*3*) les indoles-pyridines, et (*4*) les substances diverses. Les moyens de purification ainsi que la détermination des structures des substances appartenant à la catégorie (*1*) et (*2*) sont décrits dans cet article. Des preuves spectroscopiques et des synthèses ont permis d'attribuer les structures **1**, **2**, **3**, et **4** aux quatre β-carbolines simples et les structures **5**, **6**, **7**, et **8** aux quatre pyridines simples isolées.

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We recently described in a preliminary communication (1*a*) the isolation of a number of alkaloids from the bark of a West African tree, *Nauclea diderrichii* (*Sarcocephalus diderrichii*). We were able to demonstrate the co-occurrence of indole alkaloids, presumed to be derived from tryptamine or tryptophan, and pyridine alkaloids that are probably of monoterpenoid origin, and evidence was presented that some of the alkaloids isolated, referred to as indole-pyridines, contained structural units derived from both of these classes and were of considerable structural novelty. In the present discussion attention will be focussed on the simpler alkaloids to which structures have been confidently assigned; experience with these alkaloids has helped us to discern in the more complex alkaloids (1*b*) structural features which appear to reveal a pattern, probably related to the biogenetic origins of these secondary plant metabolites (1*c*), which can then be applied to generate or test suggestions regarding the structures of some of the more complex alkaloids.

Isolation and Classification of Constituents

Although the genus *Nauclea* L. (*Sarcocephalus* Afzel. ex R. Br.) of the family Rubiaceae is widely distributed in tropical areas (2), contains species with reputations in native medicine (3), and is botanically closely related (4) to species from which constituents of considerable chemical interest have been isolated, it appears to have attracted remarkably little phytochemical interest (3).

We have investigated the bark of *N. diderrichii*, obtained from Nigeria, and isolated a considerable number of constituents, principally alkaloids, of chemical interest. Isolation procedures used were based on those commonly used for the extraction of alkaloids: typically, the dried, ground bark was extracted repeatedly with cold methanol, the extract was concentrated under reduced pressure, the components were partitioned between dilute hydrochloric acid and chloroform, and the basic fraction was recovered from the acidic solution after it had been treated with ammonia. The crude "total bases" isolated corresponded to between 0.01 and 0.075% of the dried bark. It should be noted that included in the "total bases" were components which were later found to be non-basic and that the use of ammonia as a standard practice in the extraction and the subsequent chromatography led to questions which will be discussed subsequently (1*b*). The t.l.c. showed that the "total bases" comprised an extremely complex mixture of components, and repeated preparative t.l.c. was required to effect their separation. In some cases it was possible to purify the compounds isolated

[1]Our preliminary communication (1*a*) is considered to be Part I of this series. This account is taken from the Ph.D. thesis of D. G. Murray, University of Toronto, 1971.

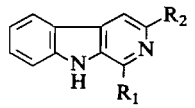
to a satisfactory level of homogeneity, but in others the very small amount of material available precluded further purification, and material that would, under better circumstances, have been considered of unsatisfactory homogeneity was studied.

In no case, however, was sufficient material available for investigation of its structure by standard methods requiring combustion analysis and rational degradation. Instead, it was necessary in every case to carry out first a careful mass spectrometric examination of the component to determine its molecular formula and to obtain any available structural information from its fragmentation pattern; other spectroscopic methods were then used, to the extent that the amount of material and its solubility allowed, in order to derive as much additional structural information as possible. On the basis of the information thus obtained, a structure was proposed as a working hypothesis with a degree of confidence that varied from case to case. It was then necessary to turn either to direct comparison with authentic material in the few cases where the compound appeared to have been described previously, or to an unambiguous synthesis of the compound when no previous account of a substance of the proposed structure could be found.

At the present time it has not been possible to carry the elucidation of structure to a satisfactory conclusion in every case, but sufficient progress has been made to allow us to see the development of a pattern in the structures that is of considerable interest and significance in the chemistry of secondary plant metabolites, and this has induced us to report our present results even in those cases where the evidence allows only a part structure to be proposed or a structural hypothesis to be advanced tentatively. It is convenient at this stage to divide the materials isolated into four categories: (*1*) simple β-carbolines, (*2*) simple pyridines, (*3*) indole-pyridines (N_3 alkaloids which appear to contain a tryptamine-derived unit and an independent pyridine unit), and (*4*) miscellaneous substances.

(1) Simple β-Carbolines

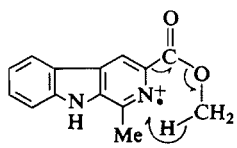
The four products (**1**, **2**, **3**, **4**) isolated that are in this category are conveniently described first since they were easily related to compounds of known structure. The identity of harman (**1**), a widely distributed and well-known alkaloid, was confirmed by comparison with authentic material. 3-Carbomethoxyharman (**2**) does not appear to have been obtained previously as a natural product, but a glycoside of the free acid has been found (5) and the ester has been synthesized from tryptophan (6); synthetic material we obtained by the same procedure (6) served to confirm the identity of natural **2**. 1-Carbomethoxynorharman (**3**) has previously been described as a natural product (7); it has also been synthesized from harman (8) and this synthesis was used to establish the identity of our natural material. 1-Carboxamidonorharman (**4**) seems not to have been described previously; its identity was established by comparison with an authentic sample derived from synthetic **3**.

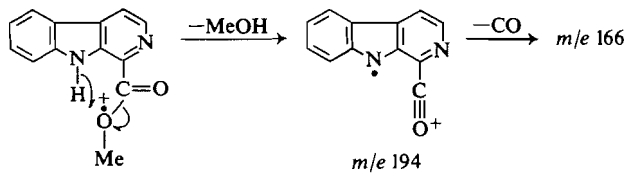


1 $R_1 = Me$; $R_2 = H$
2 $R_1 = Me$; $R_2 = CO_2Me$
3 $R_1 = CO_2Me$; $R_2 = H$
4 $R_1 = CONH_2$; $R_2 = H$

It is recognized that **4** may be an artifact derived from **3** or a related compound during the isolation process, which involved the use of ammonia at two stages. Attempts to prepare **3** and **4** via the corresponding acid chloride were abandoned in favor of Fischer esterification of the carboxylic acid and ammonolysis of the ester when it was found that the treatment of the hydrochloride of the carboxylic acid with refluxing thionyl chloride led to a considerable amount of chlorination of the β-carboline; the corresponding chlorination of a pyridine ring is known to occur (9), but under somewhat more vigorous conditions than we used.

A few features of the mass spectra of these β-carbolines (10) deserve comment since they have some diagnostic value. The base peak at M − 58 (5) in the spectrum of **2** does not correspond to an ion in the normal fragmentation pattern of an ester of an aromatic acid (11) (for which peaks are also present), and is best accounted for on the basis of a process such as:



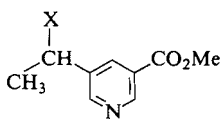


SCHEME 1

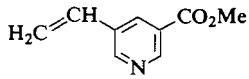
Accurate mass measurements and metastable ion evidence support this proposal. A corresponding fragmentation would be expected for **3** and the M − 58 peak is, indeed, prominent; however, the base peak is at M − 60 and apparently arises from a process involving the indole nitrogen (see Scheme 1). A significant peak is visible at *m/e* 194, and accurate mass measurements and metastable ion evidence are in accord with the sequence shown. Fragmentation of **4** apparently follows closely related pathways leading to peaks corresponding to M − CONH, M − NH_3 and M − $CONH_3$; accurate mass measurements and metastable ion evidence again provided support for the proposal, and replacement with deuterium of the hydrogen atoms bonded to nitrogen produced the anticipated spectral shifts.

(2) Simple Pyridines

Four alkaloids (**5**, **6**, **7**, **8**) in this class were isolated, and their structures were assigned on the basis of their spectroscopic properties and confirmed by synthesis. Despite the structural simplicity of the compounds, only two of them (**6** and **8**) could be found in the literature, and these appear in a patent (12) without description of their physical characteristics or origin.



5 X = OMe
6 X = OH
7 X = NH_2



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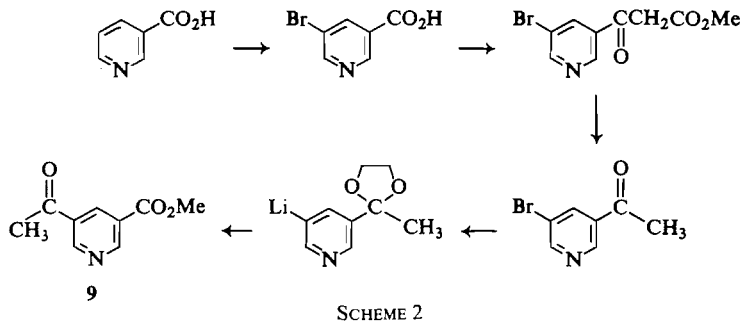
In each case the molecular formula was determined from the mass spectrum; the parent ion of **7** was not observed but its formula was determined by accurate mass measurement of fragment ions (M − 1 and M − 15). The spectrum of each of the alkaloids (**5**, **6**, **7**) with a saturated side-chain showed significant M − 1 and M − 15 peaks, the latter providing the base peak, which are attributed to fragmentation with loss of the methine hydrogen or methyl group of the side-chain. The normal aromatic ester fragmentation (11) is less clearly visible in these spectra, but in the spectrum of **8** it represents the dominant pathway and intense M − 31 and M − 59 peaks are observed.

The i.r. spectrum of each alkaloid showed a peak at 5.80 μ (with a shoulder at 5.82 μ), and the n.m.r. spectrum showed a methyl singlet near τ 6, indicative of the carbomethoxy function assigned to the alkaloid. The i.r. and n.m.r. spectra also provided the evidence that led to the recognition of the structure of the side-chain characteristic of each alkaloid, and the appearance, in the region of the n.m.r. spectrum associated with the protons of a pyridine ring, of an AMX pattern with $J_{AM} \simeq 0$ and $J_{AX} \simeq J_{MX} \simeq 2$ Hz allowed the substitution pattern of the ring to be assigned with confidence. It was observed that the appearance of the AMX pattern in the n.m.r. was markedly affected by the condition of the sample, and the presence of a trace of acid caused loss of the fine structure, with the signals at lower field being most strongly affected; the amount of peak broadening exhibited varied considerably and unpredictably, so that in some cases it was almost negligible, while in others it was sufficient to cause the signals to be submerged in the background noise. This was of considerable practical importance both here and in later cases, since spectroscopic samples that appeared pure in other respects were frequently clearly contaminated with slight traces of acid and it became our standard practice to remove the broadening by shaking the solution of the sample in chloroform-*d* with an aqueous base. The situation is also of considerable intrinsic interest since a detailed explanation of it encounters difficulties. It appears to have been observed previously (13) but was attributed to other causes, and several studies of the spectra of pyridinium salts and the influence of acidic solvents on the spectra of pyridines have been



SCHEME 2

reported (14) but without reference to the effect of traces of acid in a solvent such as chloroform-*d*.

The structures **5**, **6**, **7**, and **8** were finally established by comparisons of natural and synthetic materials. The synthetic route (Scheme 2), which proved unexpectedly troublesome, required the preparation of the key intermediate 3-acetyl-5-carbomethoxypyridine (**9**). An obvious route to the intermediate can be drawn from 3,5-pyridinedicarboxylic acid and requires a Claisen condensation of methyl acetate at one carbomethoxy group of the diester, but this gave unsatisfactory results, even though it appears to have worked well for the ethyl ester (15); the utilization of sodium methylsulfinylmethide (16) did not prove a successful variant. The reaction of dimethylcadmium with the acid chloride of 5-carbomethoxy-3-pyridinecarboxylic acid, for which we developed a satisfactory synthesis similar to one described recently by Wenkert *et al.* (17), led to some of the required product, but the yield was unsatisfactory, and the product ketone was contaminated with substantial amounts of the carbinol resulting from further reaction of the ketone with dimethylcadmium. The most successful route to **9** started with nicotinic acid which was converted through the hydrochloride of its acid chloride to 5-bromonicotinic acid; this, after esterification, was converted to the corresponding methyl ketone by Claisen condensation (18); the ketone was then protected as the ethylene ketal, the pyridyllithium compound was formed, and this was converted to **9** by carbonation and esterification.

Sodium borohydride reduced **9** to **6**, structurally identical with the natural material. The synthetic alcohol **6** was converted by methanolysis of its mesylate to the methyl ether **5**, structurally identical with the natural alkaloid. The dehydration of **6** to **8** was successfully accomplished by treatment with phosphorus pentoxide (19) or *p*-toluenesulfonic acid and this provided material suitable for establishing the structure of the alkaloid; it can be noted, however, that yields in this reaction were variable and that polymerization of **6** was a serious side reaction in the toluenesulfonic acid procedure. Reduction by zinc and acetic acid of the oxime of **9** formed **7**, structurally identical with the natural alkaloid.

The natural materials (**5**–**8**) were obtained in small quantities as oils and, with the exception of one sample of **5**, resisted attempts to crystallize them. These materials, **8** excepted, showed optical activity but because of the small amount of material available, specific rotations could not be determined with precision and confidence and it could not be established whether partial racemization of some of the samples had occurred in the case of **6** or whether variations arose from differences in the purity of the samples and instrumental difficulties. The synthetic samples were, of course, racemic and with the exception of **7**, were obtained crystalline.

Experimental

Melting points were determined on a calibrated Thomas–Kofler micro hot stage unless otherwise specified. Unless otherwise indicated, chloroform solutions were used to obtain i.r. spectra and the wavelengths of significant absorptions are reported in μ. Methanol solutions were used to obtain u.v. spectra and the wavelengths of maximum absorption (λ_{max}) are reported in mμ followed by the extinction coefficient (ε) in parentheses. Chloroform-*d* solutions with tetramethylsilane as an internal standard were used to obtain n.m.r. spectra and chemical shifts are reported on the τ scale followed in parentheses with an indication of the multiplicity of the signal and the number of protons associated with it; an indication is given for those n.m.r. spectra which were obtained after the chloroform-*d* solution had been shaken with base, usually 10% potassium carbonate in deuterium oxide. Spectrometers used were a

Perkin–Elmer 237B for i.r. spectra, a Bausch and Lomb Spectronic 505, a Perkin–Elmer 350, or a Unicam SP.800A for u.v. spectra, and a Varian A-60, A-56/60D, T-60, or HA-100 for n.m.r. spectra (values at 100 MHz are reported unless otherwise indicated). Routine mass spectra were determined at 70 eV on a CEC 21-490 or an AEI MS-902 spectrometer and the *m/e* values of significant ions are reported followed in parentheses with a figure showing the height of the peak relative to that of the base peak (100%); accurate mass measurements were made on the AEI MS-902 spectrometer. Optical rotations were determined with a Bendix–Ericsson ETL-NPL Automatic Polarimeter Type 143A. Analytical t.l.c. procedures used pre-coated Merck silica gel F254 plates, aluminum oxide F254 type T plates, cellulose F plates, or Baker-flex aluminum oxide 1B-F sheets. Preparative t.l.c. was carried out with hand-coated plates (0.5 or 1.0 mm) prepared from Merck silica gel G or aluminum oxide G type E with 3% of an electronic phosphor (excited at 254 mμ) present, or with pre-coated Merck silica gel F254 or aluminum oxide F254 type T plates which contained an electronic phosphor. Chromatograms were examined by irradiation at 254 mμ, and also by exposure of the plates to iodine in some cases.

The *N. diderrichii* bark was obtained through the good offices of Professor D. M. James, Department of Pharmacology, University of Ibadan, Nigeria. The present work was carried out on six shipments of bark, received over a period of several years and apparently collected from trees of various ages.

Standard Isolation and Extraction Procedures

Coarsely ground dry bark was covered with methanol and allowed to soak for 24 h, the methanol was drained off, and the procedure was repeated with fresh methanol.[2] The extract obtained was filtered and concentrated under reduced pressure to about 5% of its original volume, and the material obtained from four successive extractions was pooled. The concentrate was acidified with 5% hydrochloric acid and extracted twice with chloroform. The aqueous phase was basified with 10% ammonia and extracted three times with chloroform (about one third of the volume of the aqueous solution each time). This chloroform extract was washed with water, concentrated under reduced pressure to about 5% of its original volume, and thoroughly extracted with 5% hydrochloric acid. The acidic extract was then basified with 5% ammonia and thoroughly extracted with chloroform. The final chloroform extract, after it had been washed with water and concentrated under reduced pressure provided the "total bases" as a brown syrup (about 0.05% of the starting bark).

The "total bases" were subjected to a primary separation by preparative t.l.c. using silica gel plates and elution with 84:14:1 methylene chloride – methanol–ammonia (concentrated). This separation afforded about 12 fractions, each of which was examined by analytical t.l.c. to determine the best conditions for further separation. The secondary separation was carried out by preparative t.l.c. on silica gel or alumina plates and elution by various combinations of the following solvents: hexane, benzene, ether, ethyl acetate, acetone, methanol, methylene chloride, and chloroform. Systems of particular value were 5:2:3 benzene–acetone–methanol for the more polar fractions, 7:2:1 methylene chloride–acetone–methanol for intermediate cases, and 6:4 hexane–acetone for less polar fractions. Each fraction obtained was subjected to further separations by preparative t.l.c. in the manner just described until each component was obtained in as homogeneous a condition as seemed possible.

In runs which avoided ammonia, it was replaced by 10% aqueous sodium carbonate during the extraction and by triethylamine during the separation by t.l.c.

Simple β-Carbolines

Harman (1)

Harman was obtained as a white solid, m.p. 205–229°,[3] which was identical with authentic harman, m.p. 238°; the t.l.c. behavior (four systems) and the i.r., n.m.r., and mass spectra of the two samples were compared to establish their identity.

3-Carbomethoxyharman (2)

Compound **2** was obtained, after recrystallization from methanol, as a white solid, m.p. 249–253° (dec.). Spectroscopic characteristics: i.r. 2.90 (sharp), 3.07 (broad), 5.82; n.m.r. (60 MHz) 0.95 (broad; 1), 1.16 (s; 1), 1.77 (d, $J = 8$ Hz; 1), 2.2–2.9 (complex; 3), 5.98 (s; 3), 7.18 (s; 3); mass spectrum 241 (7), 240 (29), 209 (4), 183 (16), 182 (100), 181 (35), 180 (4), 179 (8), 155 (4), 154 (13), 153 (5), 128 (5), 127 (8), 105 (17), 91 (6), 90.5 (4), 77 (14), 76 (5), 63 (4), 51 (4); metastable 138.1 (calcd. for 240 → 182: 138.0); mass measurements calcd. (found): $C_{14}H_{12}N_2O_2$, 240.08988 (240.0898); $C_{13}H_9N_2O$, 209.07149 (209.0711); $C_{12}H_{10}N_2$, 182.08440 (182.0845); $C_{12}H_9N_2$, 181.07657 (181.0764). An authentic sample of 3-carbomethoxyharman was synthesized (6) and obtained as fine needles, m.p. 250–253° (lit. (6, 5) 245°, 252–253°) and shown to be spectroscopically identical with the natural material isolated and to have the same t.l.c. behavior.

1-Carbomethoxynorharman (3)

Compound **3** was obtained as white crystals, m.p. 162–167°, by sublimation of the crude base and recrystallization of the sublimate from acetone–hexane. Spectroscopic characteristics: i.r. 2.91 (sharp), 5.77 (weak), 5.89 (strong); mass spectrum 227 (10), 226 (63), 194 (15), 169 (11), 168 (84), 167 (25), 166 (100), 165 (8), 140 (19), 139 (18), 114 (12), 113 (8), 88 (5), 84 (5), 83.5 (5), 83 (6), 63 (5); metastables 142.1 (calcd. for 194 → 166: 142.0), 124.8 (calcd. for 226 → 168: 124.8); mass measurements calcd. (found): $C_{12}H_6N_2O$, 194.04801 (194.0476); $C_{11}H_8N_2$, 168.06875 (168.0684); $C_{11}H_6N_2$, 166.05310 (166.0531). An authentic sample of 1-carbomethoxynorharman was synthesized by condensation of harman with benzaldehyde followed by oxidation with potassium permanganate in aqueous pyridine and Fischer esterification of the free acid produced; procedures used followed those in the literature (8) with minor modifications. The crystalline product, m.p. 160–166° (lit. (8) 165–166°, 168°) was spectroscopically identical with the natural material isolated and had the same t.l.c. behavior; n.m.r. 0.13 (broad; 1), 1.49 (d, $J = 5$ Hz; 1), 1.95 (d, $J = 7$ Hz, with further splitting; 1), 2.02 (d, $J = 5$ Hz; 1), 2.2–2.8 (complex; 3), 5.93 (s; 3).

[2]A referee has commented that this procedure may lead to the production of methylesters as artifacts.

[3]As with many of the components obtained in this way, insufficient material was available to allow its purification to a sharp melting point by repeated recrystallization.

1-Carboxamidonorharman (4)

Compound **4** was isolated as a crystalline solid which was sublimed and then recrystallized from methylene chloride; it was obtained as white needles, m.p. 224–226° (sealed tube). Spectroscopic characteristics: i.r. 2.84, 2.91 (sharp), 2.95 (sharp), 5.97; u.v. 214 (36 000), 240 (shoulder, 17 400), 244 (18 200), 250 (shoulder, 17 000), 270 (17 800), 298 (10 000), 366 (5900); mass spectrum 212 (16), 211 (100), 194 (27), 168 (21), 167 (21), 166 (96), 165 (9), 141 (5), 140 (19), 139 (19), 114 (14), 113 (8), 105.5 (7), 88 (6), 63 (6), 57 (6), 55 (6), 44 (8), 41 (6); metastables 178.2 (calcd. for 211 → 194: 178.4), 142.1 (calcd. for 194 → 166: 142.0); mass measurements calcd. (found): $C_{12}H_9N_3O$, 211.07456 (211.0747); $C_{12}H_6N_2O$, 194.04801 (194.0475); $C_{11}H_8N_2$, 168.06875 (168.0684); $C_{11}H_6N_2$, 166.05310 (166.0532). An authentic sample of 1-carboxamidonorharman was synthesized by dissolving the corresponding methyl ester, **3**, in methylene chloride and stirring the solution for a prolonged period (2–3 weeks) at room temperature with concentrated aqueous ammonia. This afforded material, m.p. 227–229° (sealed tube) identical in spectra and t.l.c. behavior with the natural material; n.m.r. 1.58 (d, $J = 5.5$ Hz; 1), 1.84 (d, $J = 7.5$ Hz, with further splitting; 1), 1.89 (d, $J = 5.5$ Hz; 1), 2.2–2.9 (complex; 3).

Simple Pyridines

Members of this group were obtained as oils (**5** subsequently crystallized) and their structures were deduced from their spectroscopic characteristics. Authentic (racemic) samples of these compounds were synthesized and identities were established by comparison of the t.l.c. behavior (at least four systems) and the i.r., n.m.r., and mass spectra of the natural and synthetic materials.

3-Acetyl-5-carbomethoxypyridine (9)

3-Acetyl-5-bromopyridine was prepared from nicotinic acid essentially by the method of Bachman and Micucci (18) and converted to its ethylene ketal (95% yield) by treatment of a refluxing solution of the ketone in benzene with excess ethylene glycol and a catalytic amount of *p*-toluenesulfonic acid in a Dean–Stark apparatus for 1 day. The product, after sublimation and recrystallization from methylene chloride, formed thick, colorless plates, m.p. 77–78°.

Anal. Calcd. for $C_9H_{10}NO_2Br$: C, 44.29; H, 4.13; N, 5.74; Br, 32.74. Found: C, 44.10; H, 4.27; N, 5.59; Br, 33.22.

A solution of the ketal (11.5 g; 47 mmol) in 60 ml of dry tetrahydrofuran was slowly added to a solution of *n*-butyllithium (about 220 mmol) in 90 ml of hexane diluted with 100 ml of tetrahydrofuran and maintained in a bath at −40° under dry nitrogen. The temperature was allowed to rise to −15° over 15 min, and the reaction mixture was poured on to 500 g of freshly crushed Dry Ice which was kept agitated during this period and for a further 5 min. The brown residue which remained after the solvent and excess Dry Ice had evaporated was partitioned between chloroform and 10% aqueous ammonia. The aqueous phase was acidified with concentrated hydrochloric acid, washed with chloroform, neutralized with concentrated ammonia, and evaporated to dryness, the last traces of moisture being removed under vacuum (≃1 mm). The residue was ground to a powder and treated for 24 h with 700 ml of refluxing methanol containing 3% of hydrogen chloride. The reaction mixture was cooled to about 35°, and about 600 ml of the solvent was removed under reduced pressure with no increase in temperature; 800 ml of 5% hydrochloric acid was then added to the solution at room temperature and the acidic solution was allowed to remain for 30 min (to hydrolyze the dimethyl ketal formed during the esterification). The solution was then cooled, made basic with concentrated ammonia, and thoroughly extracted with chloroform. The extract afforded a brown solid (3.3 g; about 39% yield) which was taken up in 400 ml of boiling hexane, a small amount of insoluble residue was removed, and the solution was evaporated to dryness. The product, **9**, was recrystallized from water and obtained as white needles, m.p. 90–92°.

Anal. Calcd. for $C_9H_9NO_3$: C, 60.33; H, 5.06; N, 7.82. Found: C, 60.08; H, 5.35; N, 7.99.

The i.r. 5.79, 5.91; n.m.r. (60 MHz) 0.55 (d, $J = 2$ Hz; 1), 0.62 (d, $J = 2$ Hz; 1), 1.15 (3 peaks, 2 Hz spacings; 1), 5.97 (s; 3), 7.30 (s; 3); mass spectrum 180 (5), 179 (42), 165 (10), 164 (100), 148 (18), 137 (10), 136 (33), 120 (10), 104 (18), 78 (13), 50 (12), 43 (34).

3-Carbomethoxy-5-chlorocarbonylpyridine

This pyridine was prepared by the partial hydrolysis of methyl dinicotinate and treatment of the acid formed with thionyl chloride. A solution of potassium hydroxide (1.2 g; 22 mmol) in 20 ml of methanol containing 2 ml of water was added dropwise over 10 min to a stirred solution of dimethyl 3,5-pyridinedicarboxylate (4.00 g; 20.5 mmol) in 60 ml of methanol, and the solution was stirred at room temperature for 3 days. Solvent was removed under reduced pressure and the white residue was partitioned between water and methylene chloride (600 mg of starting material was recovered from the organic phase). The aqueous solution was evaporated to dryness under reduced pressure, and the white residue was ground to a coarse powder and refluxed in a dry atmosphere for 6 h in 150 ml of benzene containing 30 ml of thionyl chloride. Removal of the solvent and unchanged thionyl chloride under reduced pressure left a white residue and trituration of this with a solvent (dry benzene or ethanol-free chloroform) afforded a solution of the required acid chloride that was used in subsequent reactions. Evaporation of the solvent (benzene) left a solid residue (3.0 g) from which a crystalline sample of the acid chloride was obtained, m.p. 74–79° (lit. (17) 78°) after recrystallization from hexane; i.r. (methylene chloride) 5.66, 5.78; n.m.r. (60 MHz) 0.48 (d, $J = 2$ Hz; 1), 0.51 (d, $J = 2$ Hz; 1), 1.00 (3 peaks, 2 Hz spacings; 1), 5.97 (s, 3).

Treatment of a solution of the acid chloride in benzene with excess dimethylcadmium converted it to the ketone **9**, but yields were unsatisfactory and substantial amounts of the corresponding carbinol were also obtained.

3-Carbomethoxy-5-(1'-hydroxyethyl)pyridine (6)

A solution of sodium borohydride (102 mg; 2.7 mmol) in 10 ml of methanol was added with stirring over a period of 3 min to a solution of the ketone **9** (800 mg; 4.5 mmol) in 35 ml of methanol cooled to 5°. The reaction mixture was stirred at 5° for 5 min and at room temperature for 30 min, and 200 ml of 5% hydrochloric acid was added. After 30 min the solution was basified with ammonia and extracted with methylene chloride. The extract afforded a residue (692 mg; about 86% yield) which, when recrystallized from acetone, yielded the alcohol **6** as stout, colorless crystals, m.p. 53–56° (*p*-nitrobenzoate, m.p. 139–142°).

Anal. Calcd. for $C_9H_{11}NO_3$: C, 59.66; H, 6.12; N, 7.73. Found: C, 59.81; H, 6.11; N, 7.77.

The i.r. 2.78 (sharp), 2.97 (broad), 5.80, 5.82 (shoulder); u.v. 216 (7600), 267 (2450), 274 (shoulder); n.m.r. 0.91 (d, $J = 2$ Hz; 1), 1.21 (d, $J = 2$ Hz; 1), 1.61 (3 peaks, 2 Hz spacings; 1), 4.93 (q, $J = 7$ Hz; 1), 6.03 (s; 3), 6.17 (broad; 1), 8.46 (d, $J = 7$ Hz; 3); mass spectrum 181 (22), 180 (6), 167 (10), 166 (100), 164 (10), 150 (16), 138 (47), 134 (22), 106 (20), 104 (18), 78 (18), 77 (13), 59 (10), 51 (15), 50 (10), 43 (18).

This material was identical by the criteria listed above with the natural material; samples of natural **6** from two different lots of plant material showed $[\alpha]_D^{26}$ +23° (*c* 2.5, MeOH) and $[\alpha]_D^{25}$ +14° (*c* 2.2, MeOH).

3-Carbomethoxy-5-(1'-methoxyethyl)pyridine (5)

The synthetic alcohol **6** (1.67 g; 9.2 mmol) was converted to its mesylate by treatment for 3 days at −12 to −15° with 2.4 ml of methanesulfonyl chloride (31.6 mmol) in 40 ml of dry pyridine. The mesylate, which was obtained as a gummy solid (2.36 g), was refluxed in 25 ml of anhydrous methanol for 2 h. The reaction mixture was concentrated to an oil, treated with 150 ml of 5% aqueous potassium carbonate, and extracted with methylene chloride. The extract afforded a product (1.53 g) which was the desired ether **5** contaminated with about 15% of the starting alcohol **6**. By preparative t.l.c. (silica gel, 92:8 methylene chloride–methanol) the ether **5** (981 mg, 55% conversion) was obtained substantially pure; after sublimation and recrystallization from hexane it formed white needles, m.p. 43–46°. An analytical sample was obtained by resublimation of this product.

Anal. Calcd. for $C_{10}H_{13}NO_3$: C, 61.52; H, 6.71; N, 7.18. Found: C, 61.47; H, 6.78; N, 7.19.

The i.r. 5.80, 5.82 (shoulder); u.v. 216 (8300), 266 (2600), 273 (shoulder); n.m.r. (60 MHz) 0.77 (d, $J = 2$ Hz; 1), 1.19 (d, $J = 2$ Hz; 1), 1.65 (3 peaks, 2 Hz spacings; 1), 5.53 (q, $J = 7$ Hz; 1), 6.00 (s; 3), 6.70 (s; 3), 8.51 (d, $J = 7$ Hz; 3); mass spectrum 195 (4), 194 (5), 181 (12), 180 (100), 174 (3), 164 (17), 138 (6), 134 (5), 104 (10), 87 (8), 78 (6), 77 (8), 59 (12), 43 (19).

This product was identical by the criteria listed above with the natural material; natural **5** was obtained as an oil, $[\alpha]_D^{26}$ +50° (c 5.0, MeOH). A sample of natural **5** crystallized on prolonged standing at −15°, and sublimation and recrystallization of this from hexane afforded white needles, m.p. 47–48°.

3-Carbomethoxy-5-vinylpyridine (8)

A mixture of the synthetic alcohol **6** (817 mg; 4.5 mmol), phosphorus pentoxide (6.4 g; 45 mmol), and *m*-dinitrobenzene (590 mg) in 200 ml of xylene was stirred and refluxed for 1.75 h with exclusion of moisture. The reaction mixture was cooled in an ice bath and the supernatant liquor was separated by decantation from the gummy residue that remained. Both the liquid phase and the residue were thoroughly extracted with 10% hydrochloric acid to which ice had been added. The combined acid extracts were washed with benzene, cooled, basified with concentrated ammonia, and extracted with methylene chloride. The extract was concentrated to a syrup which was subjected to separation by t.l.c. (silica gel, 95:5 methylene chloride–methanol). The dehydrated product (110 mg; 15% yield; variable) was obtained as a syrup which was recrystallized from chilled hexane. (A procedure using *p*-toluenesulfonic acid in place of phosphorus pentoxide also provided **8** but it was contaminated with a polymer that appeared to result from transesterification of **6**.) The product (**8**) was obtained as sticky needles which melted over the range 50–75°. Spectroscopic characteristics: i.r. 5.80, 5.82 (shoulder), 6.13; u.v. 215 (15 500, end absorption), 241 (7400), 283 (1800); n.m.r. (60 MHz) 0.89 (d, $J = 2$ Hz; 1), 1.21 (d, $J = 2$ Hz; 1), 1.66 (3 peaks, 2 Hz spacings; 1), 3.23 (dd, J values 18, 11 Hz; 1), 4.07 (d, $J = 18$ Hz; 1), 4.53 (d, $J = 11$ Hz; 1), 6.03 (s; 3); mass spectrum 164 (14), 163 (100), 162 (3), 133 (11), 132 (98), 131 (11), 104 (63), 77 (27), 51 (29); mass measurement calcd. (found): $C_9H_9NO_2$, 163.06333 (163.0634).

This product was identical by the criteria listed above with the natural material **8**, which was obtained as an oil.

3-Carbomethoxy-5-(1'-aminoethyl)pyridine (7)

The ketone **9** (1.00 g; 5.6 mmol) was converted to its oxime by adding a solution of hydroxylamine hydrochloride (1.56 g; 22.4 mmol) and sodium carbonate (1.21 g; 11.4 mmol) in 6 ml of water to a solution of the ketone in 10 ml of methanol and heating the mixture on a steam bath for 1 h. Dilution with water and extraction with methylene chloride afforded the oxime (53% yield) which was recrystallized from methanol and obtained as white needles, m.p. 169–171°.

Anal. Calcd. for $C_9H_{10}N_2O_3$: C, 55.66; H, 5.19; N, 14.43. Found: C, 55.79; H, 5.29; N, 14.49.

The i.r. 2.80 (sharp), 3.04 (broad), 5.79, 6.14 (weak); n.m.r. (60 MHz) 0.76 (d, $J = 2$ Hz; 1), 0.83 (d, $J = 2$ Hz; 1), 1.42 (3 peaks, 2 Hz spacings; 1), 6.00 (s; 3), 7.68 (s; 3); mass spectrum 195 (11), 194 (100), 178 (60), 163 (84), 162 (13), 147 (10), 136 (11), 135 (20), 122 (24), 120 (11), 119 (10), 104 (14), 78 (11), 50 (12), 42 (18).

Zinc powder (13 g; 200 mmol) was added over a period of 2.25 h to a stirred solution of the oxime (576 mg; 3.0 mmol) in 50 ml of glacial acetic acid containing 6 ml of water. After the mixture had been filtered and the residue washed with 100 ml of dilute acetic acid, the filtrate and washings were diluted with 400 ml of water, made basic with concentrated ammonia, and thoroughly extracted with methylene chloride. The amine **7** (491 mg; 75% yield) was obtained from the extract as a syrup with the following spectroscopic characteristics: i.r. 3.01 (broad), 5.82, 5.80 (shoulder); u.v. 217 (10 700), 268 (2950); n.m.r. (60 MHz) 0.99 (d, $J = 2$ Hz; 1), 1.21 (d, $J = 2$ Hz; 1), 1.66 (3 peaks, 2 Hz spacings; 1), 5.72 (q, $J = 7$ Hz; 1), 6.03 (s; 3), 7.83 (broad; 2), 8.56 (d, $J = 7$ Hz; 3); mass spectrum 180 (2), 179 (6), 166 (22), 165 (100), 149 (7), 138 (10), 107 (7), 106 (7), 105 (8), 104 (6), 78 (8), 77 (5), 44 (25); mass measurements calcd. (found): $C_9H_{11}N_2O_2$, 179.08205 (179.0828); $C_8H_9N_2O_2$, 165.06640 (165.0663); $C_8H_9N_2O$, 149.07149 (149.0718); $C_7H_8NO_2$, 138.05550 (138.0556).

The *p*-nitrobenzoyl derivative was obtained as white needles, m.p. 154–157° (phase change 100–110°).

Anal. Calcd. for $C_{16}H_{15}N_3O_5$: C, 58.35; H, 4.59; N, 12.76. Found: C, 58.64; H, 4.80; N, 12.75.

Synthetic and natural **7** were identical by the criteria listed above; the natural material was an oil which was optically active, $[\alpha]_D^{25}$ +27° (c 0.34, MeOH), but this value may contain a substantial error because of the small amount of sample available.

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