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# CONSTITUENTS OF *CATHA EDULIS*

## ISOLATION AND STRUCTURE OF CATHIDINE D

M. CAIS, D. GINSBURG* and A. MANDELBAUM
Department of Chemistry, Technion-Israel Institute of Technology, Haifa, Israel

and

ROGER M. SMITH
School of Natural Resources, The University of the South Pacific, Suva, Fiji

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**Abstract**—Work which has recently appeared on the structures of Celastraceae alkaloids in addition to physical and chemical evidence adduced with respect to cathidine D permits formulation of structure **7a** or **7b** for this component of *Catha edulis*.

*Catha edulis*, better known as Khat, a bush-like plant of East African origin and a member of the Celastraceae family, was brought to the Arabian peninsula during the Middle Ages and was subsequently transplanted to Israel by Yemenite Jews. It has long been used to brew an extract with stimulant properties. Chewing of the leaves creates a similar effect. In certain countries, the plant is classified under local narcotic laws.[1]

Attempts have been made to isolate the active constituents of *Catha edulis*. Several authors have isolated an alkaloid which was named cathine.[2] Stockmann[3] added to this a description of two additional alkaloids named cathinine and cathidine. However, only solubility data and colour reactions but no other physical constants are given for any of these products. Wolfes[4] later repeated Beiter's work[2b] and obtained cathine in crystalline form. This was shown to be identical in all respects including optical activity, with (+)-*nor-pseudo*ephedrine. This substance was again isolated by Paris and Moyse[5] but paper chromatography of the crude basic fraction showed three spots apparently corresponding to alkaloids. Paper electrophoresis by the same authors showed the presence of at least five alkaloids but no attempt to isolate them was described.

More recent work claims that cathine is the only alkaloid in *Catha edulis*[6] whilst other work showed the presence of additional alkaloids by thin-layer chromatography.[7]

In addition to the unsatisfactory chemical situation described above, there are conflicting reports on the pharmacological action of extracts of *Catha edulis*.[1]

We have therefore decided to investigate the constituents of *Catha edulis* with the view of isolation of alkaloids additional to cathine and determination of their structure and pharmacological activity. This paper reports our extraction and isolation procedures and the results obtained. Our success in isolating additional alkaloids from *Catha edulis* stems, as described in the Experimental, from the use of more concentrated acid (*ca.* 2N) than is usually employed for the extraction of the alkaloids from an organic extract. These alkaloids are practically insoluble in dilute acid since they are extremely weak bases. It is therefore not surprising that previous workers who used water or dilute acid in the extraction procedures, could not isolate these weak bases.

Our extraction procedure permitted isolation of material corresponding to Stockmann's "cathidine". This comprised at least 4 components designated A, B, C, D in order of elution during column chromatography.

Cathidine D is a colourless, crystalline material which melts at 219–222° and is optically active, $[\alpha]_D$ +74°. The molecular formula is $C_{32}H_{37}O_{11}N$ (based on elemental analysis and a molecular weight of 611 by mass spectrometry).

The UV spectrum of cathidine D exhibits absorption in ethanol at $\lambda\lambda_{max}$ 229, 258(sh), 264, 273(sh), 283(sh) nm; log $\epsilon\epsilon_{max}$ 4·25, 3·60, 3·58, 3·53, 3·18.

Microanalysis showed the presence of C-Me but no OMe or NMe groups. The presence of at least one C-Me group is indicated by the existence of an intense M-15 peak in the mass spectrum of cathidine D. Alkaline hydrolysis gave a saponification equivalent of 154 which corresponds to four ester groups. Acidification of the saponified mixture followed by steam distillation showed the presence of three moles of volatile acids in the distillate and one mole of a nonvolatile acid in the distillation residue. The UV spectrum of the distillate was found to be identical with that of a solution of benzoic acid and the value of $\epsilon_{224\,nm}$ corresponded to 1 mole of benzoic acid. In addition the steam distillate was found to contain two mole equivalents of acetic acid. The latter was also confirmed by the isolation of acetamide from the ammonolysis of cathidine D. Conclusive quantitative proof for the presence of two equivalents of acetic acid was obtained by NMR measurements on the sodium acetate isolated from the distillate. One molar equivalent of nicotinic acid was then isolated from the non-volatile residue.

Thus eight O atoms in cathidine D are accounted for in ester groups and the single N atom is present in a nicotinic acid residue, providing an explanation for the feebly basic properties of the alkaloid.

Two out of the three remaining oxygens were shown to be present in alcoholic OH groups. The appearance of two bands, at 3565 and 3480 $cm^{-1}$, in the IR spectrum of cathidine D (in $CCl_4$), unchanged at high dilution, demonstrates intramolecular H-bonding between these OH groups. The acetylated and benzoylated derivatives of cathidine D show a single IR absorption band at 3540 $cm^{-1}$. This indicates that at least one of the OH groups in cathidine D is tertiary and therefore does not undergo facile acylation.

Cathidine D reacted with exactly one mole equivalent of lead tetraacetate and the crude reaction product (93% yield) did not show any IR absorption band in the OH region.

Steam distillation of the reaction products did not yield any volatile carbonyl compounds indicating that cathidine D contains only two vicinal OH groups, neither of which

is primary. The lead tetraacetate oxidation product was shown to be a keto-aldehyde. Hence one of the two vicinal OH groups in cathidine D is tertiary and the other secondary. Furthermore, the formation in this reaction, of only one product (in 93% yield) of a molecular formula $C_{32}H_{35}O_{11}N$ (elementary analysis; MW from mass spectrum) implies that the OH groups in question are attached to C atoms which form part of a ring system.

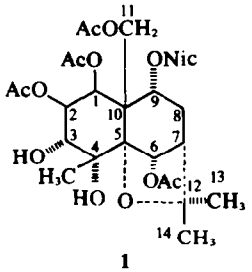
By elimination, the eleventh O atom is considered to be part of an ether function. No Cotton effect is measurable in the wavelength range corresponding to ketones (400–280 nm), neither in methanol nor in methanolic hydrochloric acid. Cathidine D gives no reaction with 2,4-dinitrophenylhydrazine and therefore it does not seem likely that the IR carbonyl absorption bands in the 1750 $cm^{-1}$ region are due, in addition to the proven ester CO groups, to a ketonic or aldehydic CO group. On the other hand, one or more of the strong absorption bands at 1100, 1090 and 1020 $cm^{-1}$ may be due to an ether function.

The evidence adduced permits one to regard cathidine D as a tetra-ester of the polyol $C_{15}H_{26}O_7$. Such a polyol would require the presence of three double bonds and/or rings. The absence of any hydrogen absorption upon attempted micro-hydrogenation of cathidine D impels one to arrive at a tricyclic structure for the polyol $C_{15}H_{26}O_7$.

As will be seen below, many recent papers on *Celastraceae* alkaloids point to the great structural similarities between them and cathidine D.

Beroza[8] had earlier shown that alkaloids obtained from *Tripterygium wilfordii* were esters of a polyol $C_{15}H_{26}O_{10}$ whilst Reichstein and Santavý[9] had shown that alkaloids of *Evonymus europeae*, likewise a plant of the *Celastraceae* family, were also esters of this polyol. On hydrolysis all of these alkaloids yielded substituted nicotinic acids.[10]

A stride forward in the eventual formulation of the structure of cathidine D resulted from the isolation and characterization of maytoline and maytine which not only stem from *Maytenus ovatus* Loes., a member of the *Celastraceae* but also contain an unsubstituted nicotinic acid esterified to a hydroxylic group in the corresponding polyols maytol, $C_{15}H_{26}O_8$ and 3-deoxymaytol, $C_{15}H_{26}O_7$.[11] An X-ray crystallographic investigation of maytoline methiodide led to elucidation of the molecular structure.[12]



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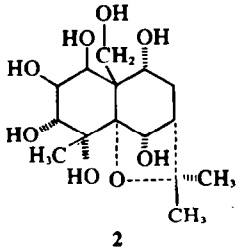
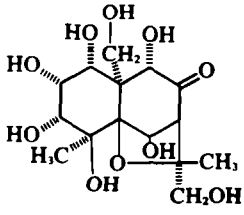


2

Maytoline is thus **1** based on the polyol nucleus **2**. Other work on evonine and evonoline showed that these were based on the respective polyols $C_{15}H_{24}O_{10}$, **3** and $C_{15}H_{24}O_9$, **4**.[13] Further work related to evonine included deacetylevonine which is based on the polyol $C_{15}H_{24}O_{10}$.[14] The absolute configurations of **1–4** were not determined and the formulations quoted are those given by their authors.

A series of papers by Hirata elucidated the structures of evonine and *neo*evonine from *Evonymus Sieboldiana*,[15,16] and of wilfordine and alatamine from *Evonymus alatus*. The latter two are based, respectively, on the polyols $C_{15}H_{26}O_{10}$, **5** and $C_{15}H_{24}O_{10}$. The X-ray structural elucidation of bromoacetyl*neo*evonine monohydrate permitted the establishment of the absolute configuration of this compound, and implicitly of the polyol **5**.[17] On this basis, the polyol $C_{15}H_{24}O_{10}$[18] is presumed to have the absolute configuration exhibited by the enantiomer of **3**, formulated above, and indeed perhaps *all* of the compounds described in this paper have the absolute configuration corresponding to **5**.



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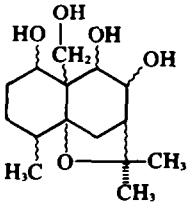
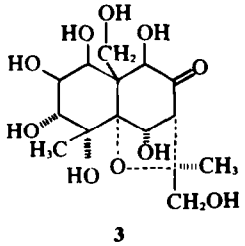


6

The isolation of malkanguniol, a $C_{15}H_{26}O_5$ polyol, **6**, from *Celastrus peniculatus* Willd., is germane to our discussion. This appears to have a similar sesquiterpenoid skeleton but configurational assignments were not made.[19] Another paper mentioning malkanguniol and two related ester alkaloids celapanin and celapanigin appeared close on the previous one's heels.[20] It is not clear how the configuration given for the ether bridge anchored at C-5 and C-7 can be correct.[20] Another paper giving a wealth of structural data rapidly followed.[21]

Finally we must mention a recent paper dealing with the structure of cathidine but the name cathidine is used for a mixture: "Cathidine is a mixture of polyalcohol esterified with different amounts of acetic acid, benzoic acid, trimethoxybenzoic acid, evoninic and nicotinic acid. The polyol is identical with a reduction product of evonine and the basic compound of evonimine".[22] Perhaps this holds for "cathidine" thus defined but this mixture cannot, then, include cathidine D which is based on the polyol $C_{15}H_{26}O_7$, not $C_{15}H_{26}O_{10}$. One of us (M.C.) had pointed out to Professor Spiteller (as indeed mentioned in Ref. 22) the potential relationship between alkaloids of *Catha edulis* and those of the maytoline or evonine types.



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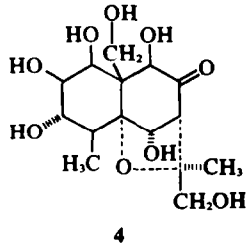


4

We had been aware of papers appearing in this field (*cf* Ref. 14, footnote 9) and of the possibility of fitting cathidine D into a sesquiterpenoid framework related to structures **1–5** but stagnation continued until one of us (A.M.) received a letter[23] from Dr. R. M. Smith who was to become our co-author. On the basis of the following arguments and analogies we now suggest that cathidine D has the structure **7a** (preferred) or **7b**.

Overall, the NMR spectrum of cathidine D closely resembles that of maytoline. Of the five quaternary Me groups (seven in maytoline) those at $\tau$ 8·60, 8·46, 8·34 are

ascribed to the two geminal Me groups, the one at C-4, and the high field $OCOCH_3$ group attached to C-1. The last is analogous to the C-1 acetate of maytoline whose Me protons exhibit a signal at $\tau$ 8·35. The high field signal in our case is presumably due to the same reason as in maytoline, shielding through the diamagnetic influence of the neighboring C-9 nicotinoyl group.[11] The second acetate signal in cathidine D is at $\tau$ 7·88.

As shown by the NMR data the configurational array at C-1, C-2, C-3, C-4 appears to be identical in maytoline and in cathidine D. The latter exhibits signals at $\tau$ 6·30 (d, J = 3·5 Hz, 1H), 4·00 (d, J = 3·5 Hz, 1H), 4·25 (m, 2H), 6·60 (bs, 2 OH), very similar to those of maytoline: $\tau$ 6·40 (d, J = 3·5 Hz, C-3), 4·09 (d, J = 3·5 Hz, C-1), 4·40 (t, J = 3·5 Hz, C-2), 6·32 (bs, OH).[11] The C-11 methylene protons in cathidine D are at $\tau$ 4·99(s) but in maytoline at $\tau$ 5·04, 5·60 (ABq, J = 13 Hz) and that at C-9 in cathidine is at $\tau$ 4·25 (m) and for maytoline $\tau$ 4·51 (bd, J = 7·5 Hz).[11]

We prefer formulation **7a** in which C-2 carries the benzoate and C-11 the acetate rather than **7b** but this is not proven. There would be more potential interference between the freely rotating nicotinoyl group at C-9 and the freely rotating benzoyloxy group were this at C-11 than if the latter were located at C-2 (and an acetoxy group at C-11). It must be admitted, however, that if there were a benzoate at C-2 one might expect the proton signal at C-3 to be at higher field than it does in fact appear and at higher field than in maytoline due to the diamagnetic influence of the aromatic ring.

Cathidine D and indeed many *Celastraceae* alkaloids, like many other natural products blissfully ignore the tenets of conformational analysis. Thus, whether C-2 bears a benzoate (**7a**) or an acetate (**7b**), the ester is axial as are the large angular group at C-10 and the larger (Me) of the two groups at C-4.

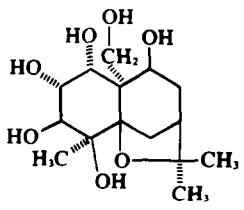
It is clear from the preparation of a keto-aldehyde **8** by lead tetraacetate oxidation of cathidine D that there is a free OH group at C-3, vicinal to the tertiary alcohol at C-4. The bond between C-3 and C-4 is cleanly broken (93% yield). The NMR spectrum of the oxidation product fully supports the above deduction. The highest-field methyl signal is at $\tau$ 8·61. This *must* be one of the geminal methyls as the C-4 Me is now adjacent to a CO group (see below). Perhaps this Me is the one which can find itself proximate to the nicotinoyl group during one phase of its circumvolutory dangling (note that it has been invoked also with respect to the C-1 acetate). In the case of maytoline the highest field Me signal is a $\tau$ 8·46 singlet corresponding to 6H which presumably belongs to the *gem*-dimethyl group.[11] A second Me signal of the keto-aldehyde is at $\tau$ 8·33 whilst those belonging to the Me groups of the two acetates appear at 7·98 and 8·14. The higher-field one (at C-1) is now presumably less shielded by the C-9 nicotinoyl group as C-1 is now part of an aliphatic chain which no longer possesses the rigidity of the erstwhile ring in cathidine D and it may thus more readily escape the sphere of influence of the C-9 ester. The lowest-field Me signal is at $\tau$ 7·62 as this belongs to $COCH_3$. The CHO signal of the keto-aldehyde is at $\tau$ −0·20, downfield from that normally found.[24] This is not surprising as this is attached to an adjacent oxygen-bearing C atom.

Of course, both NMR spectra contain the low-field signals exhibited by the benzoate and nicotinate. In cathidine D the aromatic protons of the benzoate appear at $\tau$ 2·50(m), 1·93 (dd, J = 6·2 Hz);[25] for the nicotinate $\tau$ 2·60 (C-5'), 1·60 (d, J = 8 Hz, C-4'), 1·11 (d, J = 5 Hz, C-6'), 0·63 (s, C-2').[26]

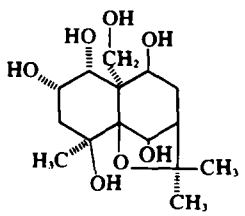
We cannot "transfer" an ester group from C-1 or C-2 without disrupting the C-1, C-2, C-3, C-4 array which we have discussed above when comparing the NMR spectra of maytoline and cathidine D. Further, we have observed the interaction between the groups at C-9 and C-1. The presence of the two doublets with J = 3·5 Hz points to the presence of three adjacent CH groups. We cannot place within the agarofuran skeleton an ester group in which one or another of the CHOR signals would not be further split by an adjacent proton. 8,9-Diols of $\alpha\alpha$, $\beta\alpha$ and $\alpha\beta$ configurations have shown $J_{8,9}$ to be 5·2, 0 and 10 Hz, respectively, and $J_{7,8}$ to be 3 Hz in all three cases.[20] Had there been a C-6 ester group in cathidine D (such equatorial groups exist in other *Celastraceae* alkaloids with singlets for the appropriate proton) the multiplet at 4·25 could hardly be appropriate for the corresponding proton.

We can rule out the presence of an ester group at C-13 or C-14 on the basis of mass spectral fragmentation of cathidine D. We observe besides $M^+$, *m/e* 611, the ion $[M-CH_3]^+$, *m/e* 596. This is readily obtained because of the formation of a stable oxonium ion, *m/e* 596. A similar oxonium ion is in fact the base peak in the mass spectrum of the octa-acetate of the polyol $C_{15}H_{26}O_{10}$, whose molecular ion is $M^+$, *m/e* 702. The fragment lost by the molecular ion corresponds to $CH_2OCOCH_3$ and the base peak mentioned, $[M-CH_2OCOCH_3]^+$ corresponds to *m/e* 629.[21] We do not observe the loss in cathidine D of either $CH_2OCOCH_3$ or $CH_2OCOC_6H_5$ or $CH_2OCONic$.

In all of the *Celastraceae* alkaloids which are esters of a polyol containing seven O atoms or more, there are only

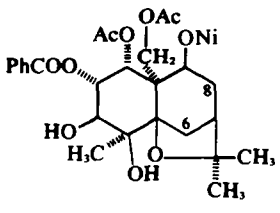


**9**

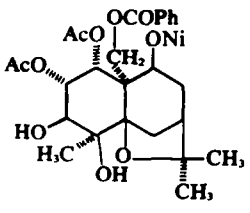


**10**

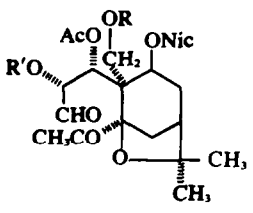
two which do not have a common relative configuration for one complete ring including C-1 to C-4 and the junctions to the other rings. The exceptions are deoxy-maytol, **10**, (which has no OH group at C-3 in contrast to maytol, **2**, and cathol, **9**, the polyol corresponding to



**7a**



**7b**



**8a**: R = COMe, R' = COPh
**b**: R = COPh, R' = COMe

cathidine D), and the polyol **4** which lacks the tertiary-OH at C-4. Maytol, **2**, and the polyols corresponding to evonine, wilfordine and alatamine are even more highly oxygenated. They contain a C-6 OH but carry extra oxygen at C-8 and C-15 (*cf.* **3**, **5**).

Thus the structure and relative configuration of cathol **9** appears to be firmly based by analogy to other polyols of the *Celastraceae* but no unequivocal decision may be made, in view of the present unavailability of the alkaloid, between formulations **7a** or **7b** for cathidine D itself.

## EXPERIMENTAL

*Catha edulis* was collected in the Embu district of Kenya under the supervision of Dr. P. J. Greenway, Botanist in charge of the East African Agriculture and Forest Research Organization.

*Extraction.* A large scale extraction was carried out through the courtesy of Doctors Haber and Hofmann of Abic, Ltd., Ramat Gan. Ground dry leaves of *Catha edusis* (30 kg) were mixed with water (50 l) and the liquid separated by centrifuging. After 3 additional treatments in the same way, 10% $Na_2CO_3$ aq. (30 l) was added and after thorough mixing the liquid was removed by centrifuging. The plant material was then continuously extracted with benzene and finally with chloroform.

After removal of the benzene from the corresponding extract (steam bath at water pump vacuum), ether (1·5 l) was added. Strong shaking (4 hr) yielded a viscous soln. Shaking this soln with 2N HCl (4 hr) gave an emulsion which was separated by removal of the ether at the water pump without heating, into an aqueous phase and a solid. The same procedure was repeated 4 times with the dark-green solid. Each time the acidic phase was filtered and addition of 4N NaOH precipitated a grey solid. The five fractions gave 8·28, 11·77, 2·07, 4·65 and 2·29 g of solid, respectively.

*Isolation.* The first 3 fractions were combined (22·1 g), dissolved in chloroform and the turbid soln was filtered through Celite in order to remove a suspension of inorganic material. After removal of the solvent at the water pump a dark yellow solid was obtained (19 g). This was redissolved in chloroform and chromatographed on a column of silica gel (600 g, 630 mm high; 45 mm diam), the fractions (25 ml) being collected by an automatic fraction collector. The results obtained are summarized in Table 1. Thin layer chromatography was used to identify the components in each fraction.

The material obtained from fractions 169–282 (5·3 g) was rechromatographed on silica gel (450 g; 500 mm column ht; 45 mm diam) but the polarity of the eluent was varied more slowly. Although the weight differences in various fractions indicated some separation, all of the fractions (total of 1050) still appeared to contain at least 3 components, as could be shown by TLC. All that was accomplished by this method was a slight change in the relative ratio of the components.

The main fractions from the above silica gel chromatographic procedure eluted by $CHCl_3$ (99·5)–MeOH (0·5) (2·65 g) were dissolved and chromatographed on active basic alumina (120 g; column ht 200 mm; diam 35 mm), 25 ml fractions being collected by an automatic fraction collector. The results are summarized in Table 2.

Rechromatography on basic alumina of fractions 35–42 which contained a mixture of cathidines B and C effected no further separation.

*Cathidine D* (from fractions 135–200) was crystallized from MeOH, m.p. 214–217°. The analytical sample had m.p. 219·5–222° (from MeOH). $(\alpha)_D$ +74° ($c$ = 0·5 in $CHCl_3$). (Found: C, 62·95; H, 6·18; N, 2·40; O, 29·01. M.W. 611 (mass spectrometric). $C_{32}H_{37}NO_{11}$ requires: C, 62·83; H, 6·10; N, 2·29; O, 28·78%. M.W. 611).

*Isolation of cathidine A.* As may be seen from Table 1, fractions 25–48 contain mainly cathidine A. The material contained in these fractions appeared to be one product by TLC. *Cathidine A*, m.p. 73–75°, is an amorphous solid which could be purified by precipitation from its soln in benzene by the addition of light petroleum.[27] (Found: C, 64·14; H, 6·66; N, 2·29; O, 26·70). These analytical figures could fit a number of empirical formulae.

Cathidine B and cathidine C were purified by TLC of the appropriate fractions from column chromatography. *Cathidine B* appeared by TLC to consist of one product. It had m.p. 112–118° (softening begins at 107°) but cathidine C was shown by TLC to be a mixture of alkaloids.

*Isolation of hydrocarbons.* The residue from extraction of the plant material with MeOH was allowed to stand with $CHCl_3$ for one week. The solid was removed and the green soln was chromatographed on a column of basic alumina (Merck). Elution with benzene gave a colourless solid, m.p. 56-60°. Attempted recrystallization from benzene-EtOH did not improve the m.p. (Found: C, 85·38; H, 14·44. "$(CH_2)_n$" requires C, 85·63; H, 14·37%). Mass spectrometry showed the solid to be a mixture of odd-numbered hydrocarbons $C_{33}H_{68}$, $C_{31}H_{64}$, $C_{29}H_{60}$ (peaks at $m/e$ 464; 436; 408, respectively) accompanied by trace amounts of even-numbered hydrocarbons in the same range, $C_{34}H_{70}$, $C_{32}H_{66}$, $C_{30}H_{62}$ (peaks at $m/e$ 478, 450, and 422, respectively).

*Saponification of cathidine D.* Cathidine D (197 mg; 0·322 mmol) was heated under reflux in a 0·925 N KOH in 90% aqueous MeOH (5 ml) for 6 hr. A blank consisting of 5 ml of the same soln without cathidine D was heated under reflux for the same period. After cooling, both solns were titrated with 0·106 N HCl using phenolphthalein as indicator. The difference in volumes of acid required in both titrations was 12.1 ml. (Found: Saponification equiv, 154. Calc. sap. eq. for 4 ester groups, 153).

Table 1.

| Material | Wt. (g) | Eluent | Fraction No. |
|---|---|---|---|
| Unknown material | 0.069 | Chloroform | 1-24 |
| Cathidine A + traces of cathidines B, C, D | 4.40 | " | 25-48 |
| Cathidines B, C, D | 1.40 | " | 49-168 |
| " | 5.42 | Chf-MeOH (98:2) | 169-282 |
| More polar alkaloid mixture | 2.96 | " | 283-380 |
| " | 2.94 | " (90:10) | 381-489 |

Table 2.

| Material | Wt.(g) | Eluent | Fraction No. |
|---|---|---|---|
| Unknown material | 0.005 | Benzene-Chf (4:1) | 1-26 |
| " | 0 | " (3:2) | 27-34 |
| Cathidines B and C | 0.053 | " | 35-38 |
| " + traces cathidine D | 1.65 | " | 39-86 |
| Cathidines C and D | 0.230 | " (1:4) | 87-122 |
| " | 0.012 | Chf | 123-134 |
| Cathidine D | 0.251 | Chf-MeoH (98:2) | 135-200 |

0·106 N HCl (20 ml) was added to the neutral soln and the whole was distilled with steam. The distillate was collected in 6 fractions and each was titrated with 0·0865 N NaOH using phenolphthalein as indicator. The results are tabulated in Table 3.

Table 3.

| Fraction No. | Volume (ml) | Eq. of volatile acid |
|---|---|---|
| 1 | 60 | 1.05 |
| 2 | 40 | 0.83 |
| 3 | 50 | 0.40 |
| 4 | 50 | 0.35 |
| 5 | 50 | 0.19 |
| 6 | 50 | 0.05 |
| TOTAL | 300 | 2.87 |

It may be observed from Table 3 that two equivalents of acid appear more volatile than the third.

The ultraviolet spectrum of the whole distillate was identical to that of benzoic acid.[29] On the basis of $\epsilon_{224nm}$ it was calculated that 0·33 mmol benzoic acid is present in the distillate. Since this was formed from 0·322 mmol cathidine D, the latter must be a monobenzoate. The distillate, neutral to phenolphthalein was evaporated to dryness *in vacuo* on the water bath. The IR (in KBr) of the solid residue was practically identical to that of a mixture of sodium acetate-sodium benzoate (2:1).

The NMR spectrum of this mixture (in $D_2O$) showed the ratio 6:5 for protons of the Me groups of the acetate residue with respect to the 5 aromatic protons in one benzoate residue. These were the only protons in the spectrum (besides those of water).

The non-volatile residue from the steam distillation was evaporated to dryness from the steam bath *in vacuo*. Repeated extraction of the dry residue with $CHCl_3$ and MeOH permitted isolation of crude nicotinic acid hydrochloride. This was converted into the free acid whose analytical sample was obtained by crystallization from abs EtOH. The UV and IR spectra were identical to those of authentic nicotinic acid.

*Attempted microhydrogenation of cathidine D.* Cathidine D (10·5 mg) in ethanol (3 ml) in the presence of pre-reduced 10% Pd-C (20 mg) was shaken with $H_2$ in the apparatus of Clauson-Kaas and Limborg,[30] for 1 week. No reduction took place although 0·45 ml of $H_2$ should have corresponded to the presence of one double bond. The concurrent efficacy of the apparatus was tested by successful microhydrogenation of cholesteryl acetate.

*Acetylation of cathidine D.* Cathidine D (20 mg) was treated with $Ac_2O$ (0·5 ml) and dry pyridine (1 ml) and the whole was set aside overnight at room temp. After the usual workup the acetate (22 mg) was obtained as a glassy solid. Scratching gave a colourless powder m.p. 75–80°, $(\alpha)_C = +50°$ ($c = 0·22$ in $CHCl_3$), which appeared to be homogeneous by TLC. It exhibits a singlet band in the hydroxyl region of the IR spectrum at 3550 $cm^{-1}$ ($CCl_4$) as compared to a doublet in cathidine D.

*Oxidation of cathidine D with lead tetra-acetate.* Cathidine D (100 mg) and lead tetraacetate (250 mg) were dissolved in AcOH (AnalaR, 8 ml) which gave a negative chromotropic acid test for formaldehyde.[31a] After standing at room temp for 22 hr the soln was rapidly steam-distilled until 15·5 ml of distillate was obtained. The distillate gave a negative chromotropic acid test and showed the absence of any volatile CO compound in a colorimetric test with 2,4-dinitrophenylhydrazine.[31b] The residue of the steam distillation was diluted with water and was extracted thrice with chloroform (30 ml portions). The combined chloroform extracts were washed with water, the solvent was removed *in vacuo*, toluene (0·5 ml) was added and this was removed *in vacuo* to aid in removal of traces of acetic acid. A colourless solid *keto-aldehyde* was obtained (93 mg).

The analytical sample, m.p. 177–180° was obtained by crystallization from ethanol. A single spot was shown by TLC. (Found: C, 62·99; H, 5·95. $C_{32}H_{35}O_{11}N$ requires: C, 63·05; H, 5·79%). IR absorption: None in OH region.

The ultraviolet spectrum was identical to that of cathidine D.

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