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THE STRUCTURE OF TUBEROSTEMONINE

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Abstract—Tuberostemonine, the major alkaloid of *Stemona tuberosa* Lour., has been proved by chemical methods to have the novel structure Ia.

THE isolation of tuberostemonine, the major alkaloid of *Stemona tuberosa* Lour. (Roxburghiaceae) was first reported by Suzuki[1] in 1934. A base obtained from *Stemona sessifolia* Franch was investigated by Schild,[2] and this was later observed[3] to be identical with tuberostemonine. A succession of reports of exploratory investigations concerning the alkaloid ensued, mainly from the laboratories of Kondo and Suzuki. The chief conclusions drawn from these earlier investigations can be summarized as follows: The alkaloid has the empirical formula $C_{22}H_{33}O_4N$.[2, 3] It contains a tertiary nitrogen, probably incorporated in a pyrrolidine ring system,[2] and possesses two γ-lactone rings.[4] The compound is without C—C double bonds.[5] Zeisel determination indicated the absence of N-Me or N-alkyl groups.[3]

In a preliminary communication,[6] published in 1961, we have established the structure of tuberostemonine as I (a or b). With the structure of the alkaloid thus clarified, Uyeo *et al.*,[7] the following year, identified the indole (II), obtained from the palladium dehydrogenation of tuberostemonine. The isolation of a *diethyl* indole parallels our own experience in the palladium dehydrogenation of the diol III obtained from tuberostemonine in several steps.[8]

Edwards[9] later examined the NMR spectrum of the pyrrole derivative IV, using the spin-decoupling technique with a view to deciding between the structures Ia and Ib. He found that the proton on C-10 of IV gives rise to a triplet, and concluded that the structure Ia is the correct representation of tuberostemonine.

In this paper we present a full report of our chemical investigations, on the basis of which the structure of tuberostemonine was proved rigorously to be Ia. The recent X-ray studies of Uyeo *et al.*[10] confirm the formulation Ia originally proposed on the basis of chemical evidence in our 1961 communication.[6]

The alkaloid, on recrystallization from methanol, was obtained as colourless prisms, m.p. 66–68°, $C_{22}H_{33}O_4N \cdot CH_3OH$. Methanol was readily lost from the crystal structure on warming *in vacuo* and fine colourless crystals of tuberostemonine resulted, m.p. 86–88°, $C_{22}H_{33}O_4N$, $pK = 6{\cdot}4$ (60% ethanol).

The base shows no UV chromophore. The presence of two γ-lactone rings was confirmed by saponification experiments, and by the IR absorption at 1765 cm^{-1}: when the alkaloid was refluxed with slightly more than one equivalent of ethanolic

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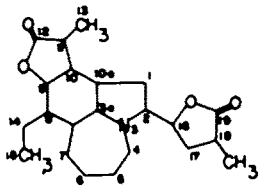
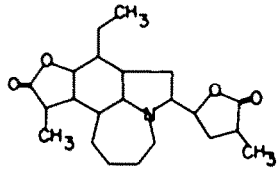
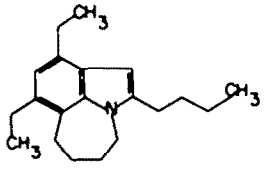


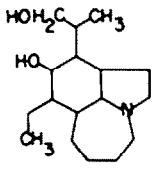
Ia



Ib



II



III

potassium hydroxide, a product resulted whose IR spectrum still showed strong lactone absorption; the latter disappeared after refluxing with two equivalents of alkali. The NMR spectrum* of the base reveals three C-Me groups. A poorly resolved triplet (three protons) centered at 9·02 τ corresponds to the Me protons of an Et group. Two sharp peaks at 8·63 and 8·77 τ (6 protons) are shown below to be two superimposed doublets rather than two unsplit C-Me's. Their chemical shift suggests that they may be located α- to the lactone carbonyls. A multiplet at 5·62 τ corresponding to two protons is attributed to the secondary γ-hydrogen of each lactone. An alternative assignment of this signal to two primary γ-hydrogens on one lactone ring was excluded by degradative studies (*vide infra*).

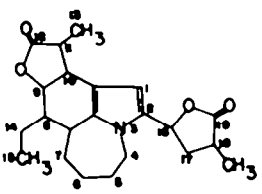
Our own investigations corroborated the conclusion of Kondo[5] that tuberostemonine contains no C—C double bonds. Thus, in addition to the two γ-lactones, the alkaloid possesses three rings, at least two of which are heterocyclic.

Mild oxidation of the base had been reported[2,11] to yield a neutral compound, $C_{22}H_{29}O_4N$ which showed a positive reaction for pyrroles by the Erlich test. The same product is produced by air oxidation of tuberostemonine on standing for several months, and may be isolated from the mother liquors of the crude alkaloid. We have obtained this pyrrole in the latter manner, and assign to it the structure IV. The presence of the lactones is manifested in the IR absorption at 1760 cm^{-1}. The UV spectrum exhibits maxima at 234 mμ (ε 10,900) and 278 mμ (ε 1500). The substitution pattern of the pyrrole was clearly demonstrated by an analysis of its NMR spectrum. A single pyrrole β-proton appears at 4·08 τ, split into a narrow doublet (J = 2·2 c/s, 1:3 split). Whereas in the spectrum of tuberostemonine the lactone γ-hydrogens appear as a two-proton multiplet at 5·62 τ, the latter protons in the pyrrole IV give rise to two one-proton signals at 4·61 and 5·39 τ.

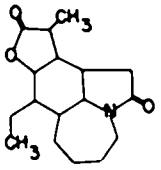
The fact that only one of these hydrogens is significantly deshielded by the pyrrole nucleus is evidence that the γ-protons are on different carbons. Clearly the oxidation product (IV) contains a 1,2,3,5-tetra-substituted pyrrole nucleus, and one of the lactone functions is attached to the latter in such a way that its γ-hydrogen is strongly

* Unless otherwise specified, all NMR spectra are measured in $CDCl_3$ with TMS as internal reference.

deshielded. The methyl protons of the ethyl group display a triplet centered at 8·96 τ, and the doublets corresponding to two C-Me groups (superimposed in the tuberostemonine spectrum) now appear, resolved, as a quartet of peaks at 8·54, 8·60, 8·67 and 8·70 τ.



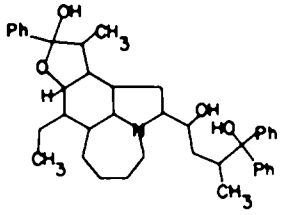
IV



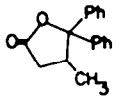
V

Further information about the environment of the nitrogen and the nature of the Me groups was extracted from a study of the permanganate oxidation[2,4,12] of tuberostemonine. This reaction was reported as giving rise to 1-methylsuccinic acid ($[\alpha]_D^{24.5}$ −20·03°) (dioxan) and a neutral compound, m.p. 135–138°. Of the formulae advanced by Schild[2] and Kondo[12] for this product, we have found that of the former author, $C_{17}H_{25}O_3N$, to be correct, and our analytical results indicate further the presence of two C-Me groups. IR absorption at 1765 and 1685 cm^{-1} confirms the presence of γ-lactone and γ-lactam functionality, respectively. The NMR spectrum of the lactam (V) exhibits a doublet for *one* C-Me at 8·70 τ, and still shows the Me protons of the Et group as a triplet at 9·03 τ. We are thus provided with convincing proof that the two deshielded C-Me groups of tuberostemonine are located at different carbons and that both are doublets. The formation of a γ-lactam, and the simultaneous loss of one of the γ-lactones as methyl succinic acid, in addition to the evidence accumulated from the pyrrole IV, indicate that in the alkaloid, one pyrrolidine α-carbon is connected to the γ-carbon of one of the lactones. It may be noted that the C_{17} lactam and methylsuccinic acid together account for the complete carbon content of the alkaloid.

The Grignard reaction of tuberostemonine opened the way to a series of valuable diagnostic experiments. Treatment of the alkaloid with excess PhMgBr furnished a crystalline product, $C_{40}H_{51}O_4N$. Three Ph groups have been incorporated, and the IR spectrum shows strong OH absorption at 3500, a low intensity band at 1605 cm^{-1}, and the absence of any CO absorption. UV maxima are at 257 mμ (ε 1900) and 276 mμ (ε 1580). The presence of 15 aromatic hydrogens is clearly demonstrated in the NMR spectrum (2·60–2·84 τ) and a signal at 5·82 τ, corresponding to one proton, can be attributed to a secondary hydrogen α to a hemi-ketal ether oxygen. In the absence of the deshielding influence of the lactone carbonyls, the three C-Me groups give rise to signals between 8·92 and 9·15 τ. The question of which lactone had reacted with only one mole of the Grignard reagent was clarified by oxidation of this product with chromium trioxide in pyridine. From the resulting acidic fraction benzoic acid and a substance, $C_{17}H_{16}O_2$, were isolated. The latter was identified as γ,γ-diphenyl-β-methylbutryolactone (VII) on the basis of spectroscopic evidence, described in the experimental section. This and later evidence lead to the structure VI for the Grignard product.

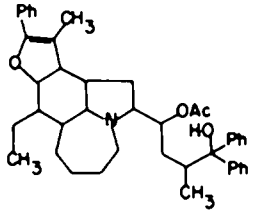


VI

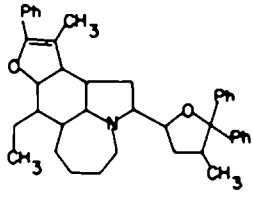


VII

Treatment of VI with acetic anhydride in acetic acid at room temperature afforded a crystalline anhydromonoacetate, $C_{42}H_{51}O_4N$. IR absorption at 1735 cm^{-1}, and a three-proton singlet at 7·85 τ in the NMR spectrum, demonstrate the presence of the O-acetyl group; a one-proton multiplet at 4·81 τ shows that the acetylated OH is secondary. As a consequence of the dehydration, one Me group is now located on a C—C double bond, and this is manifested as a sharp 3-proton singlet at 8·10 τ. The remaining two C-Me groups appear as a multiplet at 8·81–9·13 τ. The compound exhibits a UV chromophore at 280 mμ (ε 9900). Failure to isolate benzophenone upon treatment of the anhydro compound with a variety of powerful oxidizing agents proved that the elements of water have been lost by the hemi-acetal system, rather than by elimination of the C-19 hydroxyl. These data can be accommodated by the structure VIII for the anhydromonoacetate.



VIII



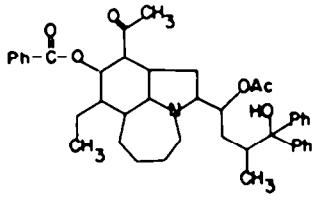
IX

Distillation of VIII in high vacuum gave rise to the crystalline ether IX, $C_{40}H_{47}O_2N$. The loss of acetic acid, suggested by the analytical result, was confirmed by the absence of acetyl peaks in the IR and NMR spectra. The substituted styrene chromophore was still evident at 281 mμ (ε 12,000). The NMR spectrum shows 15 aromatic protons between 2·61 and 2·84 τ, a 3-proton singlet at 8·12 τ for the Me on the double bond, and a 6-proton multiplet at 8·90–9·32 τ for the remaining Me groups. Ether formation is indicated by the absence of vinylic hydrogens in the NMR, and by the lack of OH absorption in the IR.

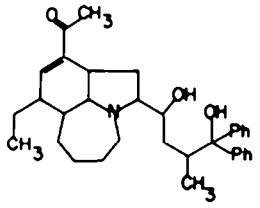
When refluxed with aqueous ethanolic hydrochloric acid, IX added a molecule of water and yielded the hemi-ketal X, $C_{40}H_{49}O_3N$, which had no styrene chromophore. X exhibited UV maxima at 252 mμ (ε 910), 258 mμ (ε 940), 263 mμ (ε 730) and 268 mμ (ε 410). Further evidence for the addition of water to the double bond of IX was provided by the disappearance of the NMR Me singlet at 8·12 τ. X could be reconverted to IX under the action of acetic anhydride.

When VIII was oxidized with chromium trioxide in acetic acid, the methyl ketone XI was obtained, which showed IR absorption ($CHCl_3$) at 3400 and 1723 (sh) cm^{-1},

corresponding to OH and acetate, respectively, and a strong peak at 1715 cm^{-1} for the ketone and benzoate. UV maxima were observed at 270 mμ (ε 1200) and 281 mμ (ε 810). The functionality of XI was clearly established by its NMR spectrum: two protons at 2·08 τ represent the ortho hydrogens of the benzoate; the 13 remaining aromatic hydrogens appear between 2·54 and 2·89 τ. The secondary hydrogen α to the benzoate, expected to absorb at 4·88 τ, is deshielded by the methyl ketone in the β-position to 4·54 τ. A one-proton multiplet at 4·91 τ is in agreement with a secondary acetate, and singlets at 7·89 and 7·92 τ account for the acetyl group and the methyl ketone. Attempted recrystallization of XI resulted in the loss of benzoic acid.



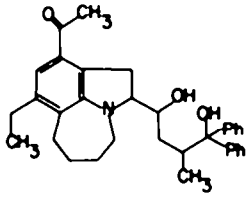
XI



XII

Elution of XI through a column of basic alumina with benzene–ether effected the elimination of the elements of benzoic acid and hydrolysis of the acetate, yielding an α,β-unsaturated ketone (XII), $C_{33}H_{43}O_3N$. XII exhibited IR ($CHCl_3$) OH absorption at 3400, and peaks at 1670 as well as 1640 (w) and 1620 (w) cm^{-1} are in accord with the CO and C—C double bond absorption of an α,β-unsaturated ketone. The UV spectrum shows a maximum at 225 mμ (ε 12,000). The β-hydrogen of the unsaturated ketone gives rise to an NMR doublet at 3·37 τ, the methyl ketone peak appears at 7·69 τ and the remaining two Me groups are shown as a multiplet at 8·82–9·15 τ.

Refluxing of XII with methanolic potassium hydroxide in the presence of air furnished basic and acidic material. The latter fraction crystallized from ethanol, and was identified as γ,γ-diphenyl-β-methylbutyrolactone (VII), identical with a sample obtained from the oxidation of VI. Chromatography of the basic fraction yielded, in addition to starting material, a pale yellow crystalline compound XIII, $C_{33}H_{39}O_3N$. The IR spectrum ($CHCl_3$) shows OH absorption at 3600 and 3400, and a peak at 1680 cm^{-1}, correctly located for an aromatic ketone.



XIII

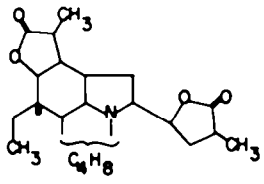
UV maxima at 252 mμ (ε 17,800), 275 mμ (sh) (ε 8700) and 354 mμ (ε 1900) represent a typical *ortho*- or *meta*-aminoacetophenone chromophore. On protonation of the nitrogen by the addition of 1N HCl, UV maxima were observed at 252 mμ (ε 12,900) and 282 mμ (ε 3200), absorption characteristic of an alkyl substituted acetophenone.[13]

The NMR spectrum of XIII reveals 11 hydrogens in the aromatic region at 2·49–2·77 τ, proving that the newly formed aromatic ring is penta-substituted. The methyl ketone peak at 7·47 τ is in agreement with an acetophenone, and a C-Me triplet (J_{AB} = 6·6 c/s) is centered at 8·82 τ, the expected position for an ethyl benzene. The remaining C-Me doublet appears again at 9·10 τ.

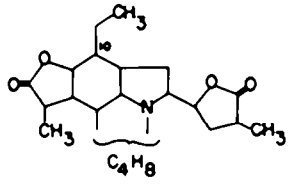
The UV evidence alone was not sufficient to differentiate between an *ortho*- or *meta*-aminoacetophenone; however, a study of the shifts of the single hydrogen on the newly formed aromatic ring in the NMR spectra[14] of a number of derivatives of XIII, helped to clarify the substitution pattern. Only a hydrogen *ortho* to the acetyl group would be expected to be significantly deshielded in the NMR spectrum of XIII in acidic medium. The smaller deshielding effect on a *meta* or *para* hydrogen would be compensated by the shielding influence of the alkyl substituents. The spectrum, taken in $CDCl_3$/HCl, exhibits, besides 10 protons at 2·78 τ, a one-proton singlet at 2·33 τ—corresponding to the predicted position for a proton *ortho* to the acetyl group.

Reduction of the CO group of XIII was effected with sodium borohydride in methanol, and afforded the corresponding triol XIV. The IR spectrum ($CHCl_3$) showed OH absorption at 3600 and 3400 cm^{-1} and the absence of CO absorption. UV maxima appeared at 254 mμ (ε 3900) and 269 mμ (ε 1850). In acidic solution the UV spectrum displayed maxima at 253 mμ (ε 1300), 269 mμ (ε 1580) and 277 mμ (ε 1580). The NMR spectrum of XIV exhibits a 10-proton peak at 2·78 τ and a one-proton singlet at 3·36 τ. Such a marked upfield shift can only arise if the hydrogen is either *ortho* or *para* to the amino group. Since it must also be *ortho* to the acetyl function, it follows that the relative position of the acetyl and amino groups is meta.

The evidence accumulated to this point allows the assignment of the partial structure XV (a or b) to the alkaloid.



XVa

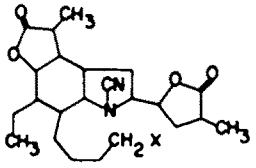


XVb

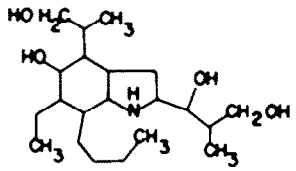
An alternative formulation, with a bridge between the N atom and C-8 or 10, and the appropriately relocated Et group, is incompatible with the properties of the aromatic derivative (XIII).

The nature of the remaining four C atoms was ascertained by a study of the von Braun degradation of the alkaloid. Kondo *et al.*[15] reported the isolation from this reaction of a substance, m.p. 200–202°. When we treated tuberostemonine with cyanogen bromide, a product XVI was obtained, $C_{22}H_{33}O_4N \cdot BrCN$, m.p. 80–85°. This compound showed a strong cyanide band at 2200 cm^{-1} in the IR, in addition to the lactone absorption at 1775 cm^{-1}. A 2-proton multiplet at 5·52 τ in the NMR spectrum corresponds to the two γ-hydrogens of the lactones. A one-proton peak at 6·16 τ was attributed to a hydrogen α to the cyanamide which is further deshielded by the β-influence of one of the lactones. A 3-proton signal centered at 6·66 τ repre-

sents the second hydrogen α to the cyanamide as well as the two protons α to the bromine.



XVI: X = Br
XVII X = OAc



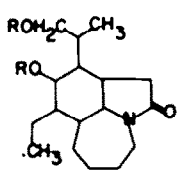
XVIII

That this assignment was in fact correct was demonstrated by converting the primary bromide into the primary acetate XVII, $C_{25}H_{36}O_6N_2$, with silver acetate in pyridine. Two of the hydrogens comprising the 6·66 τ signal are now shifted downfield to 6·03 τ as expected. Thus the secondary nature of the third carbon attached to the nitrogen is established.

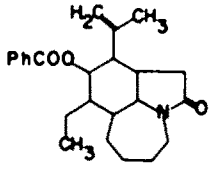
The arrangement of the three C atoms remaining unaccounted for was determined by a modified Kuhn–Roth[16] oxidation performed on XVIII, the LAH reduction product of XVI. The acids thus obtained were identified in the form of their methyl esters by VPC. Whereas tuberostemonine under similar conditions had yielded only acetic and propionic acids, XVIII gave rise to acetic, propionic, butyric and valeric acids.

This evidence supports the structure XVIII for the reduced von Braun product, and allows the assignment of the formulation I (a or b) for tuberostemonine.*

At this stage we felt that a rigorous corroboration of the structure, as well as the removal of the remaining ambiguity, could best be achieved by degradation of the alkaloid to an indole retaining the desired structural asymmetry, followed by synthesis of the latter. It has already been noted that dehydrogenation of tuberostemonine derivatives containing oxygen functionality in the side chains leads to the formation of *diethyl* indoles,[7,8] lacking the desired asymmetry. To overcome this, the lactam V was reduced with lithium borohydride, giving rise to the lactam diol XIX, $C_{17}H_{29}O_3N$; and the latter was converted to the dibenzoate XX,



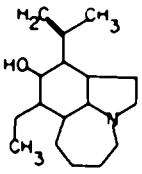
XIX: R = H
XX: R = COPh



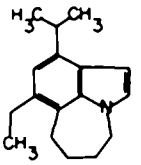
XXI

* The numbering system employed is that adopted by Edwards,[9] based on the system given for azepino 3,2,1-hi indole in Patterson *et al.* (A. M. Patterson, L. T. Capell and D. F. Walker. The Ring Index, American Chemical Society Publications, Washington, 1960).

$C_{31}H_{37}O_5N$. Pyrolysis of XX at 320–330° in a nitrogen atmosphere furnished a mixture, rich in the olefin XXI, as indicated by its NMR spectrum. The product was not isolated, but was reduced directly with LAH to XXII, which again was not subjected to further purification. XXII was dehydrogenated by heating with a Pd–C



XXII



XXIII

catalyst in a sealed tube under nitrogen at 200–230°. Neutral material was separated, and chromatography of the resulting colourless oil resulted in the isolation of the pure indole XXIII (4% overall yield from the benzoate XX). The IR spectrum (CCl_4) showed a sharp, medium intensity peak at 1165 cm^{-1} (not present in the spectrum of the corresponding diethyl indole). UV maxima occur at 227 mμ (ε 32,600), 276 mμ (ε 9100), 286 mμ (sh, ε 7900) and 297 mμ (sh, ε 5200). The NMR spectrum (CCl_4) was highly diagnostic, showing a pair of doublets at 3·27, 3·32 τ and 3·73, 3·78 τ for one α- and one β-indole hydrogen,[17] respectively. A one-proton singlet at 3·44 τ represents the single benzenoid hydrogen. The two protons on C-4, α to the nitrogen, appear as a poorly resolved triplet centered at 5·84 τ. A 5-proton multiplet extending between 6·85 and 7·54 τ can be resolved into a triplet centered at 6·95 τ and a quartet centered at 7·34 τ, and accounts respectively for the benzylic methylene protons on the 7-membered ring and those on the Et group, as well as the single isopropyl benzylic hydrogen. A 9-proton multiplet at 8·62–8·98 τ represents the three C-Me groups.

The mass spectrum of XXIII shows a molecular ion peak at *m/e* 241. The base peak at *m/e* 226 corresponds to the loss of a Me group.

Comparison with a synthetic sample of XXIII, prepared by an unambiguous route,[18] revealed complete identity of the two, as established by IR, UV, NMR and mass spectrometry, and by TLC. The m.p. of the "natural" indole XXIII was undepressed on admixture with the synthetic material. Thus, the carbon skeleton of the lactam V is confirmed, and the structure of tuberostemonine has been rigorously established as Ia. The alkaloid is representative of a novel structural class.

EXPERIMENTAL

M.ps were determined on a hot stage apparatus, and are uncorrected. IR spectra were recorded on a Perkin–Elmer Model 21 Infrared spectrophotometer, and a Perkin Elmer Infracord Model 137B spectrophotometer, using KBr pellets except where otherwise stated. UV spectra were obtained in 95% EtOH soln on a Beckman DK-2 spectrophotometer. NMR spectra were measured with a Varian Associates 56·4 Mc/s spectrometer. The mass spectra were obtained on a Hitachi Perkin Elmer RMU-6D mass spectrometer. Microanalyses were performed by Dr. F. Pascher, Mikroanalytisches Laboratorium, Bonn, W. Germany, and by Spang Microanalytical Laboratory, Ann Arbor, Michigan.

Since tuberostemonine undergoes auto-oxidation on exposure to air (*vide supra*), the alkaloid was always recrystallized immediately prior to use.

Tuberostemonine (Ia)

The alkaloid was extracted according to the procedure of Kondo *et al.*[11] The pure base, m.p. 86–88°, was obtained by recrystallization from MeOH and ether, and drying for 3 days at 60° in vacuum. (Found: C, 70·32; H, 9·21; O, 17·28; N, 3·83; C-Me, 8·81. $C_{22}H_{33}O_4N$ requires: C, 70·37; H, 8·86; O, 17·09; N, 3·74; 3 C—Me, 12·0%).

Pyrrole (IV)

The mother liquors from recrystallization of tuberostemonine furnished colourless crystals of IV, m.p. 174–177°.[11] (Found: C, 71·02; H, 7·82; O, 17·40; N, 3·87; C-Me, 8·63. $C_{22}H_{31}O_4N$ requires: C, 71·14; H, 7·87; O, 17·32; N, 3·78; 3 C-Me, 12·1%).

Permanganate oxidation of tuberostemonine[2]

A soln of the K salt of tuberostemonine was prepared by the addition of KOH (4·0 g) in water (80 ml) to the alkaloid (5·0 g) dissolved in MeOH (25 ml), and warming the mixture for 1 hr on a water bath. When cool, CO_2 was passed into the soln until the pH was between 7 and 8, and most of the MeOH was then removed in vacuum. The soln was cooled to 0° in ice-salt, and 2% $KMnO_4$ aq (500 ml) was added with efficient stirring during 4 hr. The mixture was made slightly acidic by addition of 2N H_2SO_4, and SO_2 was passed in until the brown ppt of MnO_2 dissolved. The resulting clear yellow soln was extracted thoroughly with $CHCl_3$, and the extracts were washed several times with $NaHCO_3$ aq to separate acidic material. The organic phase, after washing and drying yielded 1·06 g of yellow oil. Crystallization from $CHCl_3$-isopropyl ether afforded the hydrate of V, m.p. 128–133°. (Found: C, 66·20; H, 8·81; O, 19·93; N, 5·09. $C_{17}H_{25}O_3N \cdot H_2O$ requires: C, 65·99; H, 8·80; O, 20·69; N, 4·35%). Water of crystallization was absent in a sublimed sample; m.p. 135–138°. (Found: C, 69·92; H, 8·95; O, 16·30; N, 4·77; C-Me, 9·4. $C_{17}H_{25}O_3N$ requires: C, 70·07; H, 8·65; O, 16·47; N, 4·81; 2 C-Me, 10·3%).

The $NaHCO_3$ soln, after acidification, yielded 2·26 g of brown resin by exhaustive ether extraction. Crystallization from benzene afforded 1-methylsuccinic acid, m.p. 109–110°. (Found: C, 45·38; H, 6·21; O, 48·54. $C_5H_8O_4$ requires: C, 45·46; H, 6·10; O, 48·44%). The dimethyl ester was obtained by treatment of 1-methylsuccinic acid with diazomethane $[\alpha]_D^{24}$ −12·58°.

Reaction of tuberostemonine with phenyl magnesium bromide

Tuberostemonine (11·25 g) dissolved in dry benzene (500 ml) was added slowly to an ethereal soln of phenyl Grignard reagent, prepared from Mg turnings (3·24 g) and bromobenzene (21·7 g). The mixture was refluxed with stirring for 20 hr. It was then poured cautiously into a soln of NH_4Cl (7·5 g) in ice–water, and the mixture was extracted with benzene. The extracts were washed successively with 5% NaOH aq and water, and after drying over Na_2SO_4, the solvent was removed. Crystallization of the residue from ether afforded 11·66 g of VI, m.p. 160–166°. After repeated recrystallizations from EtOH, the product melted at 178–187°. (Found: C, 78·52; H, 8·27; O, 10·89; N, 2·48; C-Me, 6·27. $C_{40}H_{51}O_4N$ requires: C, 78·77; H, 8·43; O, 10·49; N, 2·30; 3 C-Me, 7·4%).

Chromium trioxide oxidation of VI *in pyridine*

Grignard product VI (1·40 g) was dissolved in dry pyridine (25 ml) and added to a cooled slurry of CrO_3 (3·5 g) in pyridine (50 ml). The mixture was stirred at 0° for 2 hr, and then at 25° for 16 hr, after which the pyridine was removed *in vacuo*. The residue was diluted with water and 50% NaOH aq was added; the strongly alkaline aqueous soln was then subjected to continuous ether extraction for 32 hr. Evaporation of ether yielded 505 mg of neutral material.

The aqueous soln was made acidic by addition of 20% H_2SO_4, after which continuous ether extraction afforded 235 mg of acidic product.

The neutral substance was refluxed with 5% ethanolic KOH for 1 hr, whereupon it yielded 121 mg and 331 mg of neutral and acidic fractions, respectively. The combined acidic material (567 mg) was subjected to chromatography on silicic acid (20 g). Elution with benzene gave a white foam (A) which crystallized (178 mg) from EtOH, m.p. 140–141°. (Found: C, 80·25; H, 7·10; O, 12·79; C-Me, 5·92. $C_{17}H_{16}O_2$ requires: C, 80·92; H, 6·39; O, 12·68; 1 C-Me, 5·95%). Saponification Equivalent - Found: 253; Calc. 252·3.

Elution with benzene–MeOH (200:1) yielded benzoic acid (180 mg), m.p. 121–122°, $C_7H_6O_2$, identified by spectroscopy and by comparison with an authentic sample.

(A) was identified as VII on the basis of spectroscopic evidence. IR, 1780 (lactone), 1605 cm^{-1} (w).

UV maxima, 253 mμ (ε 530), 258 mμ (ε 650), 264 mμ (ε 500) and 268 mμ (ε 320). The NMR spectrum shows a multiplet at 2·63–2·74 τ for the 10 aromatic hydrogens, and a 3-proton doublet for the C-Me at 9·07 τ (J = 6·5 c/s). It exhibits also an ABX type pattern,[14] with the quartet for the X proton centered at 6·62 τ, and peaks for the A and B protons centered at 7·52 τ (J_{AB} = 16·4 c/s). It is clear from inspection of a model of VII that the unexpectedly low position of the X proton can be due to long-range deshielding by one of the aromatic nuclei.

Acetylation of VI

A soln of VI (10·0 g) in glacial AcOH (100 ml) and Ac_2O (100 ml) was set aside at 25° for 17 hr. After evaporation to dryness *in vacuo*, trituration with isopropyl ether afforded 10·0 g of crystalline product. Recrystallization from isopropyl ether afforded VIII, m.p. 85–93° (Found: C, 79·56; H, 8·45; O, 10·05; N, 2·05; C-Me, 8·83; OAc, 5·91. $C_{42}H_{51}O_4N$ requires: C, 79·59; H, 8·11; O, 10·10; N, 2·21; 4 C-Me, 9·50; 1 OAc, 6·80%).

Pyrolysis of VIII

Anhydromonoacetate VIII (700 mg) was distilled at 260–300°/0·1 mm. The yellow glassy distillate crystallized from MeOH, affording 297 mg of crystalline ether IX. An analytically pure sample melted at 163–165°. (Found: C, 83·81; H, 7·93; O, 5·38; N, 2·38; C-Me, 7·07. $C_{40}H_{47}O_3N$ requires: C, 83·72; H, 8·25; O, 5·57; N, 2·45; 3 C-Me, 7·85%).

Hydration of IX

Ether IX (220 mg) was refluxed in EtOH (50 ml) containing 1N HCl (1·2 ml) for 2 hr. Removal of solvent, and crystallization of the residue from EtOH yielded the hydrochloride of X (205 mg). After four recrystallizations from EtOH, the product melted at 190–220°. (Found: C, 76·28; H, 7·87; O, 7·05; N, 1·95; Cl, 5·39. $C_{40}H_{49}O_3N \cdot HCl$ requires: C, 76·45; H, 8·02; O, 7·64; N, 2·22; Cl, 5·64%). Liberation of the free base X was effected by shaking a $CHCl_3$ soln of the salt with 2% NaOH. The product, after crystallization from EtOH had m.p. 135–150°. (Found: C, 81·40; H, 8·59; O, 8·06; N, 2·34; C-Me, 6·8; active H, 0·38. $C_{40}H_{49}O_3N$ requires: C, 81·18; H, 8·34; O, 8·09; N, 2·36; 3 C-Me, 7·6; 1 active H, 0·169%).

Chromium trioxide oxidation of VIII *in acetic acid*

To an ice-cooled soln of VIII (2·0 g) in glacial AcOH (20 ml) was added CrO_3 (2·0 g) in 70% AcOH (20 ml), and the mixture was stirred at 0° for 75 min. The reaction mixture was poured onto ice (200 g) mixed with Na_2SO_3 (5·0 g), and the product was extracted with ether. Removal of solvent and crystallization of the residue from EtOH afforded 1·4 g of the acetate salt of XI, m.p. 120–130°. The free base was obtained by shaking the ether soln with 2% K_2CO_3.

α,β-Unsaturated ketone (XII)

The acetate salt of XI (3·8 g) was converted to the free base by shaking an ether soln of the former with 5% KOH. The resulting amorphous product was chromatographed on basic alumina (120 g). Elution with benzene–ether (1:1) yielded a white foam, which crystallized (1·6 g) from MeOH. After 5 recrystallizations from MeOH, analytically pure XII melted at 105–120°. (Found: C, 79·06; H, 8·71; O, 10·10; N, 2·19; C-Me, 6·99. $C_{33}H_{43}O_3N$ requires: C, 79·01; H, 8·64; O, 9·57; N, 2·79; 3 C-Me, 8·97%).

Aminoacetophenone (XIII)

(A) *Directly from* XI. The acetate salt of XI (1·5 g) was refluxed for 3 hr in the presence of air with 5% methanolic KOH (60 ml). The solvent was then removed *in vacuo* and water was added to the residue. Extraction of the alkaline mixture with $CHCl_3$ afforded 950 mg of yellow foam, which crystallized from ether to give 264 mg of XII. Evaporation of the mother liquors, and chromatography of the residue on basic alumina (22·0 g) afforded, from the benzene–ether (17:3) eluates, a yellow foam which crystallized (80 mg) from methanol. Recrystallization from MeOH afforded the pure XIII, m.p. 185–189°. (Found: C, 79·72; H, 7·73; O, 9·40; N, 2·38. $C_{33}H_{39}O_3N$ requires: C, 79·64; H, 7·90; O, 9·65; N, 2·81%).

(B) *From α,β-unsaturated ketone* (XII). XII (700 mg) was refluxed for 12 hr in 5% methanolic KOH (50 ml) in the presence of air. The dark soln was concentrated to a small volume, and water (100 ml) was added. The mixture was extracted thoroughly with $CHCl_3$. Evaporation of the extracts, and crystallization of the residue (544 mg) afforded a small amount of starting material XII. Aminoacetophenone (XIII) was isolated from chromatography of the mother liquors on basic alumina, using benzene–ether (17:3) as eluent

The alkaline aqueous soln remaining after the $CHCl_3$ extraction was made acidic by addition of 3% HCl, and was then extracted once more with $CHCl_3$. The extracts yielded 83 mg of acidic product, which after crystallization from EtOH, melted at 139–140°. This substance was identified as VII, by comparison with a sample obtained from the oxidation of VI (*vide supra*)

Sodium borohydride reduction of aminoacetophenone (XIII)

Aminoacetophenone XIII (18 mg) was allowed to react at 25° for 20 hr with $NaBH_4$ (50 mg) in MeOH (9 ml) and water (1 ml). The solvent was removed, and the residue was dissolved in ether, and washed with water. Removal of ether furnished 17 mg of foam, which crystallized from ethanol, m.p. 173–176°.

von Braun degradation of tuberostemonine

To a soln of freshly sublimed BrCN (35 g) in dry benzene (120 ml) was added, with efficient stirring during 4 hr, tuberostemonine (10 g) in benzene (100 ml). The reaction mixture was set aside at 25° with stirring for 12 hr. The resulting soln, which in some runs deposited a crystalline product, was diluted with EtOAc, and was washed with dil HCl and water. Removal of solvent yielded 7·0 g of crystalline product. Recrystallization from benzene afforded XVI, m.p. 80–85°. (Found: C, 57·22; H, 6·80; N, 6·23; Br, 18·90. $C_{22}H_{33}O_4N \cdot BrCN$ requires: C, 57·38; H, 6·91; N, 5·83; Br, 16·64%).

Cyanoacetate XVII

A soln of XVI (1·10 g) in Ac_2O (20 ml) was added to a hot soln of AgOAc (3·0 g) in pyridine (20 ml). The reaction mixture was heated to 130° for 1 hr while passing in N_2. It was then poured onto ice, and acidified with dil HCl. The organic material was extracted with $CHCl_3$, and after washing and drying, the extracts yielded 1·1 g of dark amorphous product. Repeated crystallization from benzene afforded XVII, m.p. 145–146°. IR (KBr), 2200, 1775, 1725 cm^{-1}. (Found: C, 65·13; H, 7·92; N, 6·11; O, 21·06. $C_{25}H_{36}O_6N_2$ requires: C, 65·19; H, 7·88; N, 6·08; O, 20·84%).

Kuhn–Roth oxidation of tuberostemonine

To a soln of CrO_3 (16·8 g) in water (100 ml) was added conc H_2SO_4 (25 ml). This oxidizing reagent (62·0 ml) was added to tuberostemonine (0·5 g) in a 100 ml distillation flask, and the mixture was heated to 150° while passing in O_2. As distillate was removed, the volume was kept roughly constant by addition of water. After 4 hr, 200 ml of aqueous soln had been collected, which was continuously extracted with ether. The extracts were dried, and the ether was carefully distilled off; the residue was then treated with an excess of ethereal diazomethane. The resulting soln was analysed with a Perkin–Elmer Model 154 Vapor fractometer, using a 2 m Polypropylene glycol (UC oil LB-550-X) column at 126°, with hydrogen as carrier gas at 1 ml/sec. Authentic specimens of the methyl esters of the straight chain C_2 to C_6 carboxylic acids were observed to have retention times 1′ 42″, 2′ 42″, 4′ 16″, 7′ 35″ and 13′ 34″. The mixture from oxidation of tuberostemonine was found to contain only acetic and propionic esters.

Kuhn–Roth oxidation of XVIII

XVIII was prepared by reduction of XVI in THF with a large excess of LAH, in the usual manner. Kuhn–Roth oxidation of the oily product was performed as above. After esterification of the resulting acids, the mixture was analysed by VPC. Four peaks were observed, at 1′ 41″, 2′ 42″, 4′ 17″ and 7′ 35″, corresponding to acetic, propionic, butyric and valeric esters, respectively.

Lactam diol (XIX)

Lactam-lactone V (200 mg) was added to $LiBH_4$ (500 mg) in anhyd THF (50 ml). The resulting white suspension was stirred under reflux for 23 hr. Water was added dropwise until vigorous reaction ceased, and the mixture was then diluted to 100 ml, and extracted with $CHCl_3$. After washing and drying, the solvent was removed. Crystallization of the residue from ether–CH_2Cl_2 (ca. 20:1) afforded 191 mg colourless crystals, m.p. 163–164°. IR ($CHCl_3$), 3400, 1670 cm^{-1}. (Found: C, 69·21; H, 9·94; O, 16·52; N, 5·05. $C_{17}H_{29}O_3N$ requires: C, 69·12; H, 9·90; O, 16·25; N, 4·74%).

Benzoate (XX)

To a soln of XIX (2·015 g) in dry pyridine (25 ml), cooled in an ice-bath, was added by syringe, in a N_2 atmosphere, 9·8 g of benzoyl chloride, during 5 min. The soln became pink, and gradually darkened to a maroon colour, with development of turbidity. The mixture was allowed to attain room temp, and was

stirred under N_2 at 25° for 48 hr. Most of the pyridine was then removed by distillation *in vacuo*, the temp being maintained below 30°. The mixture was poured into sat. NaCl aq containing ice, and was extracted with ether. The extracts were washed successively with 5% H_2SO_4, water, Na_2CO_3 and water, and were then dried and evaporated to dryness. After chromatography of the residue twice on Woelm neutral grade I alumina, crystallization from CH_2Cl_2–ether afforded 2 g of XX, m.p. 169–172°. IR ($CHCl_3$): 1715, 1680, 1605 (w) cm^{-1}. NMR spectrum: 1·92–2·01 τ (4 H, m); 2·39–2·60 τ (6 H, m); 4·59 τ (1 H, s); 5·65–5·00 τ (2 H, m). (Found: C, 73·82; H, 7·38; N, 2·81. $C_{31}H_{37}O_5N$ requires: C, 73·93; H, 7·41; N, 2·78%).

Pyrolysis of benzoate (XX)

828 mg of XX under N_2 in a 25 ml flask, fitted with cold finger condenser, was immersed in a salt-bath at 320°. Heating at 320–330° was maintained for 1 hr, and the products were then dissolved in ether. Separation of benzoic acid was effected by washing with sat. $NaHCO_3$ aq and 568 mg of neutral pale yellow oil was isolated. IR (CCl_4): 1725, 1700 cm^{-1}. NMR spectrum (CCl_4): 2·02–2·16 τ (ca. 2 H, m), 2·56–2·66 τ (ca. 3 H, m), 4·73 τ (ca. 1 H, s). Vinyl hydrogens appear at 5·83–5·94 τ (m), and a peak for the allylic Me protons at 8·36 τ.

Lithium aluminum hydride reduction of neutral pyrolysis product

To the pyrolysis product (rich in XXI; 560 mg) in anhyd ether (80 ml) was added LAH (665 mg). The mixture was heated under reflux for 6·5 hr and was then set aside at 25° for 12 hr. After the addition of water, organic material was extracted with ether, and the basic product was separated by washing the extracts with 5% HCl. The aqueous soln was made alkaline by addition of Na_2CO_3, and extraction with ether furnished 350 mg of basic product, containing XXII. IR (CCl_4): 3600, 1640 cm^{-1} (w), no CO.

Indole (XXIII)

Crude XXII (345 mg), obtained above, was heated for 3 hr without solvent in the presence of 10% Pd/C (520 mg), under N_2 in a sealed tube at 200–230°. The tube was opened after 12 hr, and the product was extracted by repeated washings of the catalyst with boiling ether. After filtration, the ether soln was shaken with 5% HCl, followed by water, and dried. Removal of solvent yielded 170 mg pale brown gum. The product was chromatographed on Woelm neutral grade I alumina (10 g). Elution with light petroleum yielded more than 16 mg of pure XXIII as a colourless oil. The material darkened on exposure to air, and normal recrystallization attempts were not fruitful. However, on standing in a N_2 atmosphere below 10°, the substance gradually solidified, yielding crystals, which after washing with small quantities of MeOH, melted at 46–50°. The m.p. was undepressed by admixture with authentic synthetic indole (XXIII).

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