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HAWAIIAN PLANT STUDIES—XIV[1] ALKALOIDS OF *OCHROSIA SANDWICENSIS* A. GRAY[2]

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Abstract—Two quaternary alkaloids were isolated from *Ochrosia sandwicensis* A. Gray. One was shown to be hunterburnine α-methochloride (I), while the other has been named ochrosandwine and its probable structure is 10-hydroxydihydrocorynantheol methochloride (II). The stereochemistry of the related alkaloid huntrabrine methochloride (IV) is elaborated. Two previously isolated yellow *Ochrosia* bases were shown to be ellipticine hydrochloride and methoxyellipticine for which the structure 8-methoxyellipticine (V) is suggested. A rapid separation of alkaloids from plant material is described, which involves extraction with hot dilute acetic acid, precipitation with Mayer's reagent and conversion of the complex to the chlorides by anion exchange.

THE abundant occurrence of alkaloids in the plant family Apocynaceae and its genus *Ochrosia* has been well-documented and reviewed in the literature.[4,5] Research along these lines was greatly stimulated by the physiological activity and pharmacological potential of many of these bases, particularly those of the indole type.

Ochrosia sandwicensis A. Gray is the only endemic Hawaiian species of the genus. The tree grows to a height of ca. 25 feet and is botanically related to *O. elliptica* and *O. oppositifolia*. Its Hawaiian name is *holei*, and the ancient Hawaiians extracted a yellow *tapa* dye from the bark and roots.[6] The bark also found medicinal use in ancient Hawaii,[7] while more recent tests of the crude alkaloid extract showed hypotensive properties.[8]

Strong positive alkaloid tests with *O. sandwicensis* were first reported in 1959.[9] During the same year, Goodwin *et al.* published on the isolation of ellipticine, methoxyellipticine and a new colourless base from the leaves.[10] Ellipticine was shown to represent a novel pyridine–carbazole ring system.[11]

Isolation of the new quaternary base N(b)-methylisoreserpilinium chloride (holeinine) and of a yellow base from the bark was reported in 1961.[12]

[1] Part XIII: P. J. Scheuer and T. R. Pattabhiraman, *Lloydia*, **28**, 95 (1965).
[2] This investigation was supported by a PHS Grant RG-5095 from the National Institutes of General Medical Sciences, Public Health Service.
[3] In part from the Ph.D. dissertation of W. Jordan, University of Hawaii, 1965.
[4] R. F. Raffauf and M. B. Flagler, *Econ. Botany* **14**, 37 (1960).
[5] N. G. Bisset, *Ann. Bogor.* **4**, Part 2, 65 (1961).
[6] J. F. Rock, *The Indigenous Trees of the Hawaiian Islands*, pp. 409–414. Published under Patronage, Honolulu (1913).
[7] D. M. Kaaiakamanu and J. K. Akina, *Hawaiian Herbs of Medicinal Value*, p. 44. Board of Health of the Territory of Hawaii, Honolulu (1922).
[8] R. F. Raffauf, Private communication.
[9] C. E. Swanholm, H. St. John and P. J. Scheuer, *Pacific Sci.* **13**, 295 (1959).
[10] S. Goodwin, A. F. Smith and E. C. Horning, *J. Amer. Chem. Soc.* **81**, 1903 (1959).
[11] R. B. Woodward, G. A. Iacobucci and F. A. Hochstein, *J. Amer. Chem. Soc.* **81**, 4434 (1959).
[12] P. J. Scheuer and J. T. H. Metzger, *J. Org. Chem.* **26**, 3069 (1961).

A recent re-investigation of the leaves revealed the presence of the known[13] isoreserpiline and of possibly three additional bases, but in quantities too small for further investigation.[14]

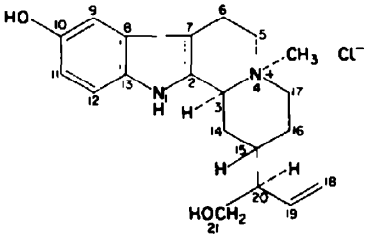
Ochrosia sandwicensis is now rather scarce and may be found in dry and elevated areas on the leeward sides of the islands. For the present work, trunk bark, mixed with some root bark, was collected in the Auahi lava fields on the island of Maui at an elevation of ca. 3000 feet and along the Pupukea road in the Koolau Range on the island of Oahu at elevations of 1000–1500 feet. Both locations furnished botanically and chemically identical plant material.

Two major extraction schemes were employed, both aiming at the separation of a water-soluble fraction rich in quaternary bases. The first scheme followed a conventional pattern. The desired aqueous fraction was chromatographed on activated carbon in order to separate the quaternary bases from sugars and undetermined coloured matter. Subsequent chromatography of the alkaloidal portion over alumina resulted in the isolation of hunterburnine α-methochloride.[15–17]

A second scheme was based on numerous pilot tests in which hot dilute acetic acid was used for extraction. Precipitation of the alkaloids with picric acid, Mayer's or Reinecke's reagents and subsequent conversion of the complexes into the chlorides by anion exchange was investigated. The results have been described in a preliminary note.[18] The preferred and most convenient method involved precipitation as Mayer's complex, anion exchange into crude chlorides and removal of tertiary bases by precipitation with ammonium hydroxide and extraction with chloroform. The resulting aqueous fraction was again rich in quaternary bases. Column chromatography on cellulose powder furnished hunterburnine α-methochloride and a new quaternary base, which has been named *ochrosandwine*.

Hunterburnine α-methochloride

Hunterburnine α-methochloride (I), together with its N(b)-β-epimer, was only recently isolated from the African species *Hunteria eburnea*.[15–17] It possesses strong



I

[13] L. A. Stoll, A. Hofmann and R. Brunner, *Helv. Chim. Acta* **38**, 270 (1955).
[14] P. J. Scheuer and S. P. Garg, Unpublished results.
[15] J. D. M. Asher, J. M. Robertson, G. A. Sim, M. F. Bartlett, R. Sklar and W. I. Taylor, *Proc. Chem. Soc.* 72 (1962).
[16] C. C. Scott, G. A. Sim and J. M. Robertson, *Proc. Chem. Soc.* 355 (1962).
[17] M. F. Bartlett, B. Korzun, R. Sklar, A. F. Smith and W. I. Taylor, *J. Org. Chem.* **28**, 1445 (1963).
[18] W. Jordan and P. J. Scheuer, *J. Chromatog.* in press.

hypotensive activity,[16,19] which had also been observed in crude extracts of *O. sandwicensis*.[8]

We isolated the base, $C_{20}H_{27}N_2O_2Cl$, in yields of 0·03% of dry bark. It crystallized from methanol, in which it was only sparingly soluble, as fine white needles, m.p. 322–324°. Solubility in dilute hydrochloric acid was so low that conventional alkaloid tests in this medium failed. Isolation from an aqueous fraction, from which it could not be extracted with chloroform even at basic pH, suggested its quaternary nature, a fact confirmed by a positive test for chloride ion and conversion into the hydroxide by anion exchange.

The UV spectrum, not affected by acid, underwent a strong but reversible bathochromic shift in base and was typical of a 2,3-disubstituted 5-hydroxyindole.

For reasons of solubility, the best NMR spectrum was obtained with the methohydroxide. It confirmed the distribution of the aromatic protons of the indole system, suggested the presence of an olefinic double bond, a hydroxymethyl and a quaternary N-methyl group and showed the absence of C-methyl.

Both the methochloride and methohydroxide could be readily hydrogenated with uptake of one mole of hydrogen. Peaks for the olefinic protons had disappeared in the NMR spectrum, while new peaks for an ethyl group suggested presence of a vinyl group in the original compound. Hydrogenation did not alter the UV spectra in various media, thus indicating that the double bond in the starting material was not in conjugation with the 5-hydroxyindole chromophore.

Acetylation of the methohydroxide furnished a diacetate as shown by NMR.

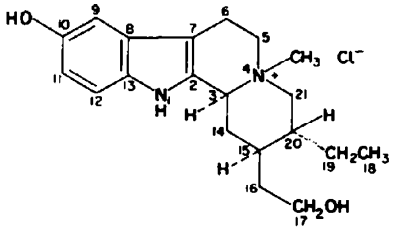
Examination of the literature revealed agreement of all these data with those published for hunterburnine α-methochloride.[17] Identity of the two compounds was confirmed by parallel IR spectra measured by the CIBA workers.

Ochrosandwine

This new alkaloid, isolated in yields of 0·08% of dry bark, crystallized from water or methanol–benzene as fine white rhombic crystals, m.p. 288–289°, $[\alpha]_D$ +85. It appeared in the separation scheme together with hunterburnine α-methochloride and gave also a positive test for chloride ion, suggesting again a quaternary base.

Combustion data of the chloride, the bromide of m.p. 274–275°, the iodide of m.p. 259–260°, the perchlorate of m.p. 250–251°, established the composition of $C_{20}H_{29}N_2O_2Cl$. A mass spectrum of the chloride confirmed the mol. wt. of 365.

We have assigned structure II to ochrosandwine on the basis of the following evidence. The spectral data for hunterburnine α-methochloride (I) and for ochrosandwine (II) suggested extensive similarities between the two compounds. The IR



II

[19] W. I. Taylor, Private communication.

spectrum of II (Fig. 1), while differing in some details, was reminiscent of the IR spectrum of I.[3] Both alkaloids gave rise to UV spectra of identical shape (2,3-disubstituted 5-hydroxyindole), including indifference to acid and a reversible bathochromic shift in base (*vide supra*). In the case of ochrosandwine, however, the base shift became irreversible when an alkaline solution was exposed to air. The UV spectrum deteriorated rapidly to a shapeless curve and reacidification did not result in the original nor any other useful spectrum. Full reversibility was achieved when the basic solution was kept under argon.

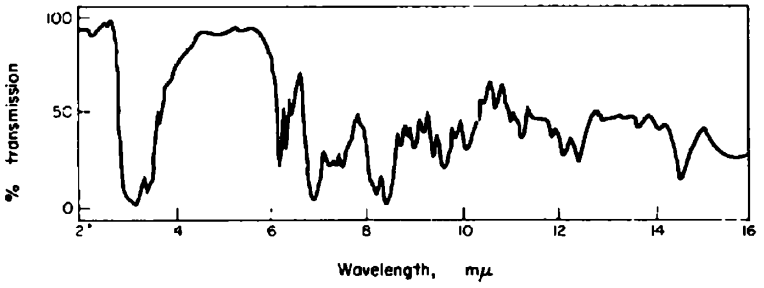


FIG. 1. IR Spectrum of Ochrosandwine (II) in KBr.

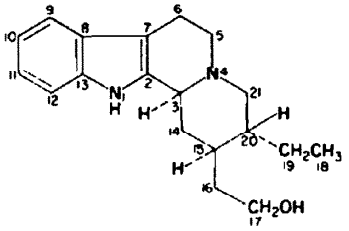
The NMR spectrum of ochrosandwine revealed the presence of an ethyl group (δ 0·9 and 1·4) and of a hydroxymethyl group (δ 3·7). Absorption at δ 6·9(C_4-H and C_6-H) and at δ 7·4(C_7-H) was consistent with a 5-hydroxyindole system. The relatively high field position at δ 2·6–2·9 for the quaternary N-methyl suggested that the group was in an axial position as part of a *trans*-fused quaternized quinolizidine system.[20] The large splitting value of the peak (20 c/s) indicated the possibility of rotational transformation within the molecule, a phenomenon reminiscent of the work on geissoschizoline.[21]

A further indication that ochrosandwine possesses a C/D *trans* ring system stemmed from the observation that this compound was very much more soluble in methanol than e.g. I, which belongs to the C/D *cis* series. We have observed this striking solubility behaviour with other known members of the two types.

A final confirmation of this point was obtained from the IR spectrum, which clearly showed the diagnostic 3·4–3·7 μ band, characteristic for a C/D *trans* compound.[22,23]

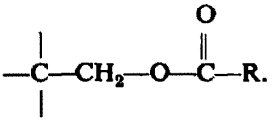
Further similarity between hunterburnine α-methochloride and ochrosandwine was demonstrated by acetylation to a noncrystalline 0,0-diacetate. Its NMR peak at δ 2·4 was assigned to the methyl hydrogens of the acetylated phenolic hydroxyl, while the peak at δ 2·1–2·2 accounted for the methyl protons of an acetylated aliphatic hydroxymethyl. That such a peak did arise from this group and not from acetylation of the indole >NH was demonstrated by Djerassi *et al.*[24] They acetylated dihydrocorynantheol (III) and showed by mass spectrometry that the affected group was the

[20] T. M. Moynehan, K. Schofield, R. A. Y. Jones and A. R. Katritzky, *J. Chem. Soc.* 2637 (1962).
[21] R. E. Moore, Dissertation, University of California, Berkeley, 1963.
[22] E. Wenkert and D. K. Roychaudhuri, *J. Amer. Chem. Soc.* 78, 6417 (1956).
[23] M. Shamma and J. M. Richey, *J. Amer. Chem. Soc.* 85, 2507 (1963).
[24] B. Gilbert, L. D. Antonaccio and C. Djerassi, *J. Org. Chem.* 27, 4702 (1962).



III

β-hydroxyethyl side chain and not the β-carboline portion of the molecule. An additional NMR peak at δ 4·1 was assigned to the methylene protons in

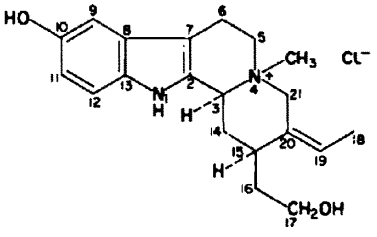


Evidence for the existence of a partial yohimbine skeleton in ochrosandwine came from aromatization experiments with selenium. Chromatography on alumina yielded two major fractions. The faster migrating one was a mixture of two closely related compounds which could neither be separated nor crystallized and had the UV spectrum of a 2-pyridylindole (alstyrine).[25–27]

The other fraction absorbed at longer wavelength and underwent a reversible bathochromic shift in base. Its IR spectrum was very similar to the one reported for alstyrine,[28] but showed a broader absorption in the 3·00–3·50 μ region. It is most likely that this material differs from the fast migrating fraction in having retained the phenolic hydroxyl. Ochrosandwine therefore belongs to the corynantheine-alstonine group of alkaloids.[25]

With the above accumulated evidence in hand it appeared that ochrosandwine might well be dihydrohunterburnine α- or β-methochloride. Direct comparison of ochrosandwine with these two compounds, however, ruled out such a simple structural relationship.

Huntrabrine methochloride (IV) was isolated by the CIBA group along with the hunterburnines from *Hunteria*, but the stereochemistry of the compound was not



IV

[25] E. Schlittler and J. Hohl, *Helv. Chim. Acta* **35**, 29 (1952).
[26] P. Karrer and P. Enslin, *Helv. Chim. Acta* **32**, 1390 (1949).
[27] P. Karrer and P. Enslin, *Helv. Chim. Acta* **33**, 100 (1950).
[28] C. Vamvacas, W. v. Philipsborn, E. Schlittler, H. Schmid and P. Karrer, *Helv. Chim. Acta* **40**, 1793 (1957).

reported.[17] An attractive structure for ochrosandwine might therefore be one of the two epimeric dihydrohuntrabrines. Such a comparison was not, however, realized experimentally, since all attempts to hydrogenate the ethylidene portion of huntrabrine failed. Uptake of hydrogen resulted in an Emde degradation, even under carefully controlled conditions.

TABLE 1. MELTING POINTS AND OPTICAL ROTATIONS OF OCHROSANDWINE AND RELATED COMPOUNDS

Compound	M.p.°	$[\alpha]_D$	Ref.
Ochrosandwine	288–289 (C)	+ 85 (M)	3
	261–262 (K)		3
Dihydrocorynantheol	272–273 (C)	+ 63 (WM)	28
methochloride	296–297 (C)	+ 101 (?)	17
Huntrabrine methochloride	285–287 (C)	+ 54 (W)	17
Corynantheidol methochloride	223–225 (C)	− 43 (WM)	28
Melinonine B chloride	311 (C)	− 15 (WM)	28
Dihydromelinonine B chloride	295–296 (C)	− 14 (WM)	28
Ochrosandwine methiodide	258–260 (K)	?	3
10-Methoxydihydrocorynantheol methiodide	263–266 (?)	?	29,30

C, evacuated capillary; K, Kofler block; M, methanol; W, water; ?, not reported.

In spite of the failure of a direct test of this structural hypothesis there exists indirect evidence that ochrosandwine is indeed one of the two dihydrohuntrabrines. Djerassi *et al.*[24] measured the mass spectrum of dihydrocorynantheol (III) and observed peaks at m/e 156, 169, 170, 184 and 297. The mass spectrum of ochrosandwine exhibited all of these peaks but they were displaced by 16 mass units in accordance with the postulated phenolic hydroxyl. A peak at m/e 269 attested to the facile loss of methyl chloride and of the β-hydroxyethyl side chain.

The case for the identity of ochrosandwine with 10-*hydroxy-dihydrocorynantheol methochloride* can be strengthened further by comparing optical rotations and m.ps of several key compounds in this series. Two known C/D *cis* alkaloids, melinonine B chloride and its dihydro derivative,[28] and the methiodide of 10-methoxydihydrocorynantheol[29,30] are included in Table 1 for comparison. Ochrosandwine, dihydrocorynantheol methochloride and huntrabrine methochloride emerge with positive rotations of comparable magnitude.

The two remaining stereochemical assignments in ochrosandwine (II) at C-15 and C-20 follow from Wenkert's C-15 rule[31] and from comparison with dihydrocorynantheol methochloride and corynantheidol methochloride. These two compounds differ only by the position of the ethyl group at C-20. Analogy with dihydrocorynantheol methochloride is demonstrated by the rotation data in Table 1.

In view of these arguments stereochemical assignments may also be made for huntrabrine methochloride (IV), which is $\Delta^{19,20}$-*dehydro*-10-*hydroxy-dihydrocorynantheol methochloride*.

[29] N. Dastoor and H. Schmid, *Experientia* **19**, 297 (1963).
[30] J. M. Ferreira, B. Gilbert, R. J. Owellen and C. Djerassi, *Experientia* **19**, 585 (1963).
[31] E. Wenkert and N. V. Bringi, *J. Amer. Chem. Soc.* **81**, 1474, 6535 (1959).

Ellipticine hydrochloride

In the course of the work on holeinine, a yellow base, m.p. 289–296° dec, yield 0·04% of dry bark, was isolated from *Ochrosia sandwicensis*. It had spectral characteristics similar to those of ellipticine and was not further investigated at that time.[12]

We purified the same compound during the present work. It crystallized as yellow needles, was readily soluble in water and gave a positive test for chloride ion. Addition

TABLE 2. COMPARISON OF THE YELLOW BASE FROM *O. Oppositifolia* AND METHOXYELLIPTICINE

	Buzas *et al.*	Present work
M.p.°	282–284	279–284
UV maxima, mμ	242, 275, 290, 335	246, 276, 292, 337
Hydrochloride, m.p.°	282–290, crystal transformation at 260	288–290, crystal transformation at 255–265
Picrate, m.p.°	268–272	273–275

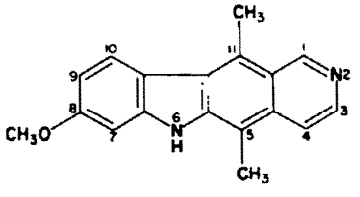
of ammonium hydroxide to an aqueous solution furnished a precipitate which could be recrystallized from methanol as yellow needles or rosettes identical in every respect with ellipticine.[10]

The yellow alkaloid was therefore ellipticine hydrochloride.

Methoxyellipticine

In their paper on a new alkaloid from *Ochrosia oppositifolia*, Buzas *et al.*[32] point out that the compound might be identical with methoxyellipticine characterized by Goodwin *et al.*[10] Both groups report the formula $C_{18}H_{16}N_2O$ and the presence of one methoxyl group. We also purified methoxyellipticine from *O. sandwicensis* and, in order to clarify the findings of the French workers, prepared the same derivatives. The significant data are shown in Table 2 and indicate that the yellow base of Buzas *et al.* was indeed methoxyellipticine.

No information was thus far available concerning the position of the methoxyl group in the molecule. The UV spectra of methoxyellipticine, 5-methoxyindole and 6-methoxyindole are shown in Fig. 2 and the corresponding fluorescence spectra in Fig. 3. Inspection of these curves suggests that methoxyellipticine possesses structure V.



V

[32] A. Buzas, M. Osowiecki and O. Schindler, *C.R. Acad. Sci., Paris* **247**, 1390 (1958).

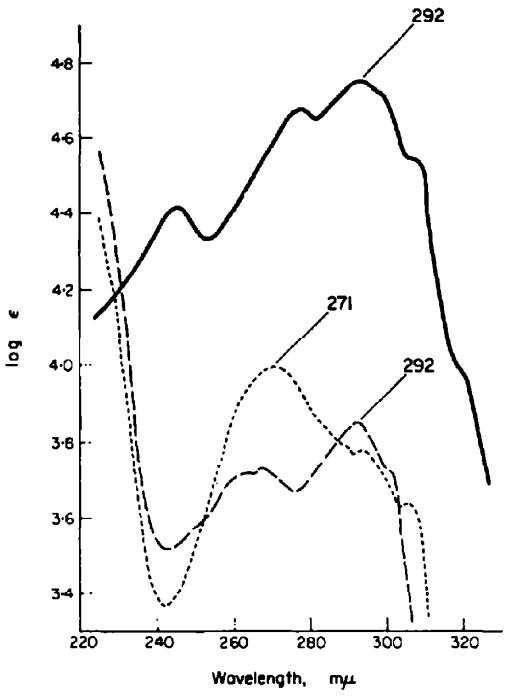


FIG. 2. UV Spectra in 95% EtOH of
methoxyellipticine ———
6-methoxyindole – – – –
5-methoxyindole . . . . . .

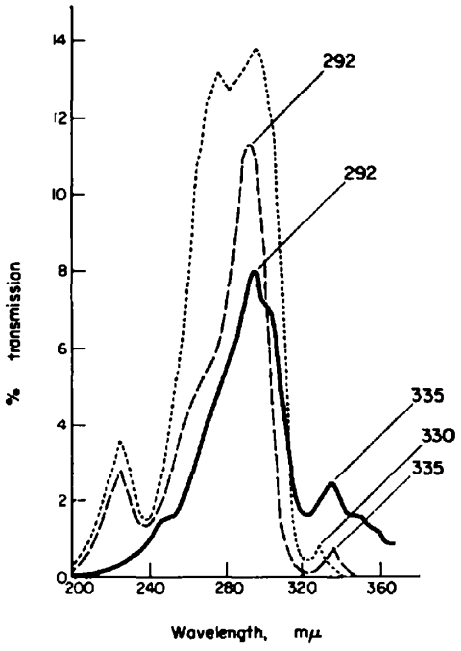


FIG. 3. Fluorescence Spectra in 95% EtOH of
methoxyellipticine ———
6-methoxyindole – – – –
5-methoxyindole . . . . . .

EXPERIMENTAL

M.ps are uncorrected. Elemental analyses were performed by Dr. A. Bernhardt, Mülheim, Germany; Dr. W. Zimmermann, University of Melbourne; and Berkeley Analytical Laboratory, Berkeley, California. UV spectra were obtained with a Beckman DK-2 instrument and are expressed as mμ (log ε). IR spectra were measured as KBr disks, using a Beckman IR-5 spectrophotometer. NMR spectra were determined with a Varian Associates model A-60; the sweep width was 500 c/s; the maxima are expressed as δ values, referring to tetramethylsilane as $\delta = 0$. Fluorometry readings were taken with an Aminco–Bowman instrument. The mass spectrum was kindly measured by Mr. A. H. Struck of Perkin-Elmer Co. Anion exchange resins used were Amberlite IRA-401 or Bio-Rad AG 1-X4, both analytical grade, chloride form and identical in performance.

Extractions and separations

The fresh bark was dried in a forced draft oven at 50° for 70 hr, ground in a Wiley mill to pass a 1-mm screen and stored in airtight containers.

Scheme A. Bark from Maui (2 kg) was continuously extracted with 8 l. MeOH (1 d), 6 l. MeOH (2 d) and 6 l. MeOH (3 d). The combined solutions were concentrated *in vacuo* to 750 ml syrup. Dilution with 1 l. water and addition of conc. NH_4OH to pH 10 gave a dark brown precipitate (106 g) rich in tertiary bases.

After filtration, the ammoniacal filtrate (2 l.) was continuously extracted with $CHCl_3$ (3 d). During this process, 0·63 g (0·03% yield) holeinine precipitated gradually from the $CHCl_3$ fraction and was removed by filtration. Evaporation of the filtrate furnished 18·2 g of a brown solid, again rich in tertiary bases.

The ammoniacal aqueous phase was concentrated *in vacuo* to 270 g of a dark and stiff syrup containing quaternary bases. One half (135 g) was diluted with water to 300 ml and chromatographed

through a column packed with a slurry of 225 g of Darco G 60 activated carbon, 135 g diatomaceous earth and 1·25 l. water. A mild aspirator vacuum was needed for good flow rates. Elution was started with 10 l. water (fractions 1–12), was continued with 3 l. MeOH (fractions 13–15) and was completed with 2 l. isopropyl alcohol (fractions 16–18). TLC suggested combination of fractions 6–18. A precipitate formed in this mixture on standing. After filtration and recrystallization from MeOH, it gave 131 mg hunterburnine α-methochloride.[16,17]

The filtrate of fractions 6–18 was evaporated to a brown glass (25·3 g) and chromatographed through a column of 550 g of Woelm neutral alumina, activity grade I. Elution required 10 l. $CHCl_3$ containing MeOH increasing from 10–30% (24 fractions). Fractions 10–24 could be crystallized and recrystallized from MeOH, MeOH–ether or MeOH–benzene and gave 128 mg of hunterburnine α-methochloride. Total recovery from this extraction: 259 mg or 0·026% of dry bark.

Scheme B. Bark from Oahu (1·2 kg) was extracted with 3·6 l. boiling 5% aqueous acetic acid (1 hr), then twice with 2·4 l. boiling water (1 hr each). The pH of the combined filtrates was adjusted to 1·0 with conc. HCl and 2·7 l. Mayer's reagent[25] added. The resulting precipitate of crude Mayer's complex was filtered off and only partially dried in air. It was stirred in 1·5 l. acetone–MeOH–water (6:2:1)[17] and non-alkaloidal insolubles discarded after filtration. The dark reddish-brown solution (2 l.) contained 144 g dissolved Mayer's complex and was passed slowly (12 hr) through a column of 450 g anion exchange resin (chloride), followed by 1·3 l. of the above solvent mixture for washing. The eluate contained 58·3 g crude chlorides. After reduction of its volume to 350 ml, water and NH_4OH were added to pH 10 and 800 ml volume. A precipitate (34·0 g) rich in tertiary bases formed and was filtered off. Extraction of the ammoniacal filtrate with 10 portions $CHCl_3$ (total 2·2 l.) removed more (1·9 g) crude tertiary bases, while the aqueous phase furnished 22·0 g of a dark solid rich in quaternary bases. This solid was heated in 250 ml MeOH and 3·07 g non-alkaloidal insolubles removed. Addition of ethyl acetate (150 ml) formed a new non-alkaloidal precipitate (0·84 g) which was also filtered off. Evaporation of the filtrate gave 18·0 g of a dark brown solid containing the crude quaternary chlorides.

A slurry of 700 g Whatman Column Chromedia cellulose powder CF 11 and 6% water in acetone was packed tightly into a glass column with a tamping rod and the crude quaternary fraction passed through it using 12 l. 8% water in acetone for elution (33 fractions).

Fractions 22–27, on crystallization from MeOH, gave 338 mg hunterburnine α-methochloride (0·028% of dry bark).

The mother liquors of fractions 24–27 were sufficiently pure (TLC) for further crystallization. Crystallization and recrystallization from water furnished 912 mg of an alkaloid designated ochrosandwine (0·076% of dry bark).

Hunterburnine α-methochloride (I)

The crystals charred on a Kofler block at 300–360° but did not melt. M.p. (evac. capill.) 322–324° dec, (lit.[17] 335°); $[\alpha]_D^{21}$ +10·1 (*c*, 0·25, 27·5% water–MeOH); $[\alpha]_D^{25}$ +44·1 (*c*, 0·60, 40% water–pyridine); λ_{max} (MeOH) 268 sh (4·05), 274 (4·08), 302 (3·78), 311 sh (3·72), λ_{min} 246 (3·54), 295 (3·76); λ_{max} (water) 274 (4·08), 297 sh (3·82), 308 sh (3·66), λ_{min} 248 (3·66); unchanged in 1 N HCl; λ_{max} (0·25 N NaOH) 269 (4·00), 323 (3·74), λ_{min} 257 (3·98), 295 (3·40); reacidification restored the neutral spectrum. (Found: C, 66·10, 66·20; H, 7·75, 7·62; O, 9·02; Cl, 9·67. Calc. for $C_{20}H_{27}N_2O_2Cl$: C, 66·19; H, 7·50; O, 8·82; Cl, 9·77%).

Hydrogenation of methochloride. Hunterburnine α-methochloride (55 mg) was dissolved in 30% water–EtOH (75 ml) and 21 mg catalyst ($PtO_2 \times H_2O$, 83·8%) added. Shaking at room temp (4 hr) under H_2 (1·7 atm) gave, after filtration and evaporation, 54 mg of a colourless glass. Crystallization from water yielded 31 mg white needles and rosettes, m.p. (Kofler block) 317–318 dec; UV spectra in different media identical with the starting material. (Found: C, 65·53, 65·73; H, 8·27, 8·42; N, 7·30, 7·58. $C_{20}H_{29}N_2O_2Cl$ requires: C, 65·83; H, 8·00; N, 7·67%.)

Hunterburnine α-methohydroxide. A column of anion exchange resin (32 ml) was converted from the chloride to the hydroxide form with 1 N NaOH and hunterburnine α-methochloride (123 mg) in 27·5% water–MeOH (100 ml) passed through it. Evaporation of the eluate gave the methohydroxide (112 mg) as a brown glass. It was hygroscopic, sensitive to air oxidation and did not crystallize, m.p. (Kofler block) 200–205°; UV spectra in different media as with the methochloride; NMR (D_2O): δ 1·4, 2·0, 3·0, 3·5, 5·2, 6·6–6·7, 7·1–7·2.

[25] B. T. Cromwell in *Moderne Methoden der Pflanzenanalyse* (Edited by K. Paech and M. V. Tracey) Vol. IV, p. 373. Springer-Verlag, Berlin (1955).

Acetylation of methohydroxide. The methohydroxide (40 mg) was refluxed under argon in pyridine (2 ml) and acetic anhydride (1 ml) for 4 hr. The product was evaporated to dryness, dissolved in water–MeOH (1:1) and passed through an anion exchange column (chloride form). Evaporation of the eluate gave amorphous material. NMR of the diacetate (D_2O): δ 2·0, 2·3, 3·2, 4·0, 5·2, 6·8, 7·1.

Hydrogenation of methohydroxide. Hunterburnine α-methohydroxide (40 mg) in MeOH (15 ml) was stirred at room temp under H_2 with 10 mg of PtO_2 for 2 hr. Filtration and evaporation furnished an amorphous product. NMR (D_2O): δ 0·8, 1·3, 2·2, 3·2, 3·6, 6·9, 7·2–7·4.

Ochrosandwine (II)

Purity of the compound was demonstrated by TLC on alumina with 20, 25, 30 and 40% MeOH–$CHCl_3$ as the liquid phase. In addition, a 90 mg sample gave three successive crops on fractional crystallization: 42 mg from water, 10 mg from water and 19 mg from MeOH–benzene. They were all identical as shown by TLC, IR and NMR. M.p (evac. capill.) 288–289° dec, m.p. (Kofler block) 261–262° dec; $[\alpha]_D^{28}$ +85·3 (*c*, 2·00, MeOH); λ_{max} (MeOH) 267 sh (3·87), 275 (3·92), 299 (3·65), 309 sh (3·58), λ_{min} 246 (3·35), 296 (3·64); λ_{max} (water) 275 (3·92), 295 sh (3·75), 306 sh (3·58), λ_{min} 248 (3·50); no shifts in 0·75 N HCl; λ_{max} (0·25 N NaOH under argon) 268 (3·71), 321 (3·47), λ_{min} 296 (3·30); reacidification under argon restored the neutral spectrum; NMR (D_2O); δ 0·9, 1·4, 2·6–2·9, 3·7, 6·9, 7·4. (Found: C, 66·09, 66·01; H, 8·44, 8·21; N, 7·28, 7·21. $C_{20}H_{28}N_2O_2Cl$ requires: C, 65·83; H, 8·00; N, 7·67%.)

Perchlorate. A sample of ochrosandwine (40 mg) was dissolved in water (3 ml) and 20% $HClO_4$ (0·25 ml) added at 50°. The white precipitate was recrystallized from 3 ml and again from 2 ml of hot water, yield, 20 mg of colourless rosettes, m.p. (Kofler block) 250–251° dec. (Found: C, 55·80, 55·77; H, 7·18, 6·93; N, 6·45, 6·39. $C_{20}H_{29}N_2O_6Cl$ requires: C, 56·00; H, 6·81; N, 6·53%.)

Bromide and iodide. Two columns of anion exchange resin (10 ml each) were converted from the chloride to the hydroxide form with 1·0 N NaOH. One of them was then converted to the bromide, the other one to the iodide form with 1·0 N HBr and HI, respectively. Two samples of ochrosandwine (25 mg each) were dissolved in 25 ml MeOH–water (2:1) and passed through the columns. The eluates were evaporated, crystallized and recrystallized from MeOH–benzene. Bromide: 24 mg of white rosettes, m.p. (Kofler block) 274–275° dec. (Found: C, 59·2, 59·3; H, 7·3, 7·4. $C_{20}H_{29}N_2O_2Br$ requires: C, 58·69; H, 7·13%.) Iodide: 20 mg of tan-coloured rosettes, m.p. (Kofler block) 259–260° dec. (Found: C, 52·3, 52·5; H, 6·5, 6·7. $C_{20}H_{29}N_2O_2I$ requires: C, 52·64; H, 6·40%.)

Acetylation with acetic anhydride–pyridine. A solution of ochrosandwine (40 mg) in pyridine (4 ml) and acetic anhydride (2 ml) was refluxed under argon for 5 hr. Evaporation gave a brown glass which was dissolved in water and passed through an anion exchange column (chloride form). It was further purified by chromatography through alumina with 10% MeOH–$CHCl_3$ as the solvent. The diacetate (21 mg) did not crystallize. NMR (D_2O): Two new peaks at δ 2·1–2·2 and 2·4.

Acetylation with ketene. A solution of ochrosandwine (38 mg) in $CHCl_3$ (25 ml) was stirred at room temp (4 hr) while ketene was admitted from a generator for 5 min periods at ½ hr intervals. The dark tar resulting after evaporation of the brown solution was separated into two fractions by repeated shaking with water–benzene (1:1) in a separatory funnel. Evaporation of the aqueous portion yielded 42 mg of the diacetate as a yellow glass. It did not crystallize and showed the same new NMR peaks as above.

Aromatization. A mixture of ochrosandwine (50 mg) and Se powder (500 mg) was heated in a test tube under argon to 280–305° (30 min) and the black residue leached with MeOH. Filtration and evaporation gave a yellow–brown glass (39 mg). Two major alkaloidal components (TLC) were separated by chromatography through neutral alumina with 30% MeOH–$CHCl_3$ as the solvent (13 fractions). TLC (alumina, 30% MeOH–$CHCl_3$) suggested combination of fractions 1–3 (double spot of R_f 0·8–0·9) and fractions 9–13 (single spot of R_f 0·5). Both combined fractions were purified by sublimation and their qualitative UV spectra in MeOH measured. Fractions 1–3: λ_{max} (neutral) 328, λ_{min} 272; λ_{max} (0·5N HCl) 299, 377; λ_{max} (0·1N NaOH) 329; λ_{max} (reacidified) 296, 375. Fractions 9–13: λ_{max} (neutral) 232, 356; λ_{max} (0·5 N HCl) 232, 356; λ_{max} (0·1 N NaOH) 243, 377; λ_{max} (reacidified) 233, 356.

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