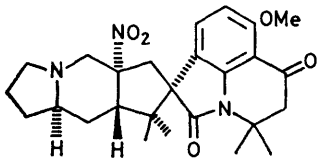
Cyclopiamines A and B, Novel Oxindole Metabolites of *Penicillium cyclopium* Westling

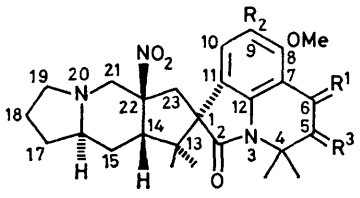
By **Roy F. Bond**, Department of Chemistry, Rand Afrikaans University, P.O. Box 524, Johannesburg 2000, Republic of South Africa
Jan C. A. Boeyens,* Cedric W. Holzapfel,* and **Pieter S. Steyn**, National Chemical Research Laboratory, Council for Scientific and Industrial Research, P.O. Box 395, Pretoria 0001, Republic of South Africa

The isolation of two new oxindole alkaloids, cyclopiamine A and B, from *Penicillium cyclopium* is described. The chemical and spectral properties of cyclopiamine B are interpreted in terms of the constitution and relative stereochemistry (2a) of the compound as determined by *X*-ray structure analysis. The enantiomeric relationship between cyclopiamine A(1) and B(2a) at C(22) is established on the basis of chemical studies.

P. CYCLOPIUM Westling is frequently encountered on stored grain and cereal products destined for human and animal consumption.[1] A toxinogenic strain of this fungus, CSIR 1082, was grown on sterilized maize meal and the toxic principles removed by a chloroform–methanol extraction. Fractionation of the toxic extract yielded two alkaloids, designated cyclopiamine A and B. These compounds together with cyclopiazonic acid accounted for the toxigenicity of *P. cyclopium*.[2,3] The same alkaloids were subsequently isolated by us from a strain (CSIR 1308) of a cyclopiazonic acid-producing fungus identified as *Penicillium urticae* Bainier.

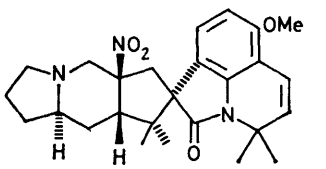


(1)



(2)

a; $R^1 = O, R^2 = H, R^3 = H_2$
b; $R^1 = O, R^2 = Br, R^3 = H$
c; $R^1 = O, R^2 = H, R^3 = D_2$
d; $R^1 = NNH_2, R^2 = H, R^3 = H_2$
e; $R^1 = H, OH, R^2 = H, R^3 = H_2$



(3)

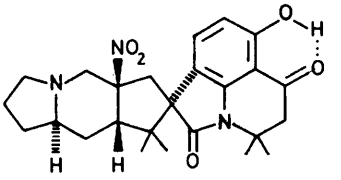
Cyclopiamines A (1) and B (2a), $C_{26}H_{33}N_3O_5$, are diastereoisomeric metabolites which exhibit a strong blue-green fluorescence upon u.v. illumination. Cyclopiamine B (2a) is a colourless crystalline compound, whereas cyclopiamine A (1) is an amorphous powder which could be converted into a crystalline hydrobromide. Chemical degradation and derivatisation of cyclopiamine B together with application of physico-chemical techniques provided knowledge on its different structural units. The final construction of the cyclopiamine B molecule and its relative stereochemistry were established by single-crystal *X*-ray crystallography. The structure and relative stereochemistry of cyclopiamine A (1) were deduced from a correlation of its physico-chemical properties with those of cyclopiamine B (2a). This paper relates the data from the chemical and physico-chemical investigations of cyclopiamines A and B, the synthesis of a model compound (8), and the *X*-ray analysis of cyclopiamine B.

Cyclopiamine B (2a) had u.v. and i.r. properties which indicated a complex aromatic moiety in the molecule. The i.r. spectrum showed strong absorption at 1 718 [C(2)=O][4] and 1 686 cm^{-1} [C(6)=O] and additionally strong bands at 1 549 and 1 370 cm^{-1} due to the aliphatic nitro-group.[5]

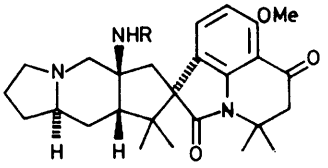
The ^1H n.m.r. spectrum of (2a) in deuteriochloroform had the following characteristics: five three-proton singlets, *viz.* at δ 1.71 and 1.40 [*gem*-dimethyl group at C(4)], 1.04 and 0.90 [*gem*-dimethyl group at C(13)], and 3.91 [methoxy-group at C(8)]; three two-proton AB patterns, *viz.* at δ 7.22 and 6.48 [C(9)–H and C(10)–H; *J* 8 Hz]; 2.80 and 2.50 [C(5)–H_2, *J* 15 Hz]; 2.92 and 2.40 [C(23)–H_2, *J* 15 Hz]; unstructured multiplets at δ 3.85 (2 H) and 2.90 (1 H), and in the range δ 2.5—1.6. In 9-bromocyclopiamine B, $C_{26}H_{32}BrN_3O_5$ (2b), the single aromatic proton C(10)–H appeared at δ 7.40. The enolisable protons in (2a) were exchanged by treatment with NaOD–CH_3OD to give dideuteriocyclopiamine B (2c); this compound also showed no absorption at δ 2.80 and 2.50.

The C(6)-carbonyl group in (2a) is relatively unreactive. No reaction occurred with hydroxylamine or hydrazine under normal conditions, but a hydrazone (2d) could be formed under drastic conditions. Potassium borohydride reduction of (2a) gave a mixture of diastereoisomeric alcohols (2e) in a ratio of 3 : 1 as indicated by ^1H n.m.r. and t.l.c. studies. The major isomer was

obtained from crystallisation of the mixture from methanol; its u.v. absorption was similar to that reported for hydroxy- and methoxy-oxindoles.[6] In the ^{1}H n.m.r. spectrum of this major diastereoisomer, the C(6)–H appeared (after deuterium exchange of the labile hydroxy-protons) as a triplet at δ 5.07 (*J* 4 Hz). In addition the compound lacked the signals at δ 2.80 and 2.50, previously assigned to the C(5) methylene protons in (2a). Dehydration of (2d) was achieved by treatment with phosphorus oxychloride in pyridine to give product (3). Compound (3) showed the presence of two olefinic protons resonating as an AB pattern at δ 6.48 and δ 5.34 (*J* 10 Hz), whereas the C(4) methyl groups became magnetically equivalent and appeared as a six-proton singlet at δ 1.63.



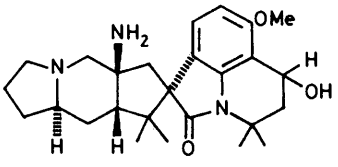
(4)



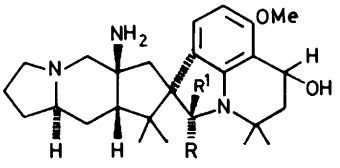
(5)

a; R = H

b; R = Ac



(6)



(7)

a; R = OH, R^1 = H

b; R = H, R^1 = OH

Demethylation of (2a) with aluminium chloride in nitrobenzene at room temperature readily furnished the phenol (4). This ready cleavage is typical of a phenolic ether *peri* to a carbonyl group.[7] Elevated temperatures were, however, required for the demethylation of (2a) in 6M-hydrochloric acid. A bathochromic shift of 13 nm in the long-wavelength absorption band on formation of the phenol (4) is in agreement with the *peri*-location of the phenolic hydroxy-group. The hydrogen-bonded C(10) carbonyl group absorbed at 1 659 cm^{-1} in the i.r. region, compared to 1 686 cm^{-1} for (2a). The newly-formed phenolic proton gave rise to a sharp signal at δ 10.58.

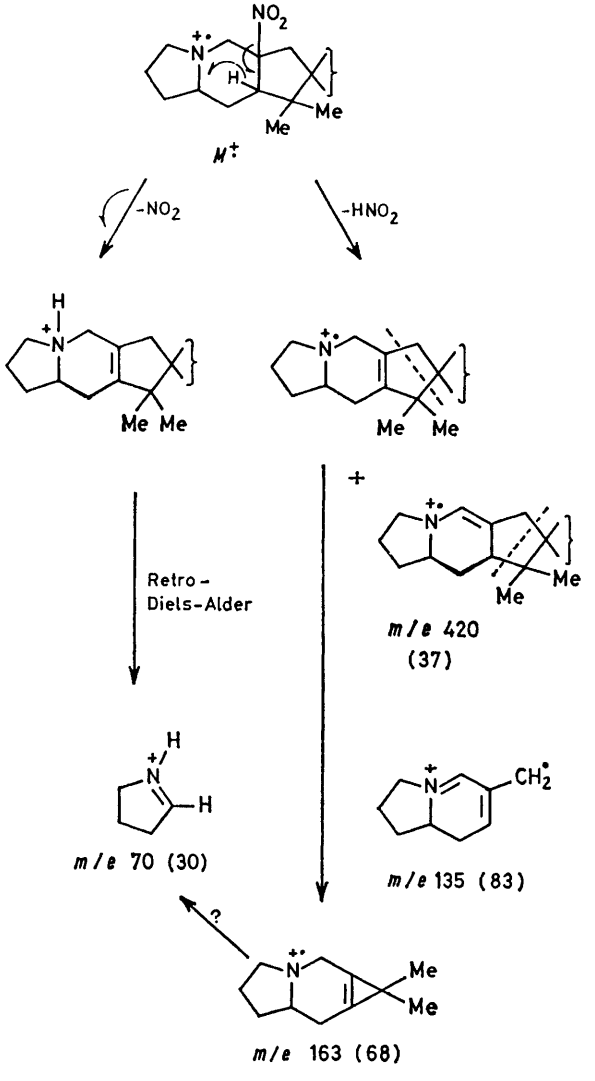
Selective reduction of the nitro-group in (2a) was achieved by catalytic hydrogenation in the presence of palladium–carbon or by reduction with sodium dithionite in aqueous methanol[8] to yield (5a). The latter upon treatment with acetic anhydride and pyridine gave the acetyl derivative (5b). Compound (5a) upon treatment with potassium borohydride gave the alcohol (6); further reduction with lithium aluminium hydride in dioxan gave the carbinolamine (7a).[9] The i.r. spectrum of (7a) lacked absorption in the carbonyl stretching region; its u.v. spectrum showed considerable agreement with that reported for 1,2,3,4,10,11-hexahydro-6-methoxy-9,11-dimethylcarbazole.[10] Addition of dilute hydrochloric acid caused a hypsochromic shift of 13 nm in the long wavelength absorption of (7a); a similar shift was exhibited by *m*-methoxyaniline upon acidification. Oxidation of (7a) with chromic acid in acetone transformed it into the starting material (5a).

Equilibration of the carbinolamine (7a) with dilute hydrochloric acid resulted in the formation of the diastereoisomer (7b). It seems likely that the initially formed carbinolamine (7a) is the product of kinetic control, whereas (7b) must be the thermodynamically preferred product. Reduction of the amide carbonyl group with lithium aluminium hydride occurs most likely *via* attack of the reagent from the least hindered side [*i.e.* the side opposite to the *gem*-dimethyl group at C(13)]. The newly-formed hydroxy-group in the resultant carbinolamine suffers severe steric interaction with one of the methyl groups on C(13). Isomerisation to the less-hindered carbinolamine (7b) may proceed through an intermediary amino-aldehyde or may involve reversible hydration of an immonium ion. After deuterium exchange of the hydroxy-protons in (7a) the singlet in the n.m.r. spectrum at δ 5.20 was assigned to C(2)–H; in (7b) the corresponding proton resonated at δ 5.60. This difference in chemical shift is in part due to the proximity of the C(2)–H to one of the C(13) methyl groups in (7a).[11]

The mass spectrum of (2a) showed a weak molecular ion at *m/e* 467. The loss of a nitro-radical and the elements of HNO_2 from M^+, which leads to the fragments at *m/e* 421 (base peak) and *m/e* 420, respectively, are characteristic of tertiary aliphatic nitro-compounds.[12] Subsequent fragmentation following electron impact was mainly directed by the charge-stabilizing nitrogen atom [N(20)]. The most abundant fragments in the low and medium mass range correspond to C_4H_8N (*m/e* 70).

$C_9H_{13}N$ (*m/e* 135), and $C_{11}H_{17}N$ (*m/e* 163) (see Scheme 1). The mass spectra of all the derivatives of cyclopiamine B invariably supported our structural assignments.

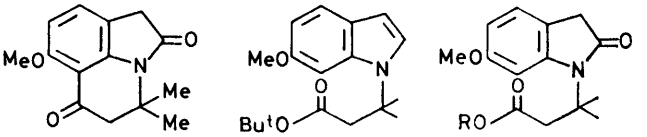
The foregoing data suggested that (2a) contained a saturated heterocyclic ring system coupled to 7-methoxy-4,4-dimethyl-1,2,4,5-tetrahydro-6*H*-pyrrolo[3,2,1-*i*,*j*]-quinoline-2,6-dione *via* the carbon atom α to the amide carbonyl group. The presence of this structural unit in (2a) was confirmed by comparison of its spectral properties with those of (8) which was prepared as follows. *N*-Alkylation of 6-methoxyindole [13] with an excess of t-butyl 3-methylcrotonate in the presence of potassium t-butoxide gave the Michael adduct (9). The yield was decreased by using more polar solvents such as dimethylformamide and t-butyl alcohol or by decreasing the excess of ester. This suggested that the equilibrium of



SCHEME 1

the reversible Michael addition does not favour the formation of the adduct, especially in polar solvents, unless the ester is used in large excess.

Oxidation of (9) with *N*-bromosuccinimide in aqueous t-butyl alcohol [14] produced a complex mixture of bromo-oxindoles and bromo-indoles. The mixture was reductively debrominated by hydrogenation in the presence of sodium acetate to give the desired oxindole



a; R = Bu^t
b; R = H

(10a) in 50% yield. Treatment of (10a) with toluene-*p*-sulphonic acid or trifluoroacetic acid gave the acid (10b) which was used for the intramolecular Friedel–Crafts acylation reactions. The acid chloride of (10b) was treated separately in various solvents with several Lewis acids. The best yield of (8) (10%) was achieved through the use of the acid chloride with aluminium chloride in dichloromethane. The u.v. spectral data of (8) were identical to those of cyclopiamine B (2a). Our deduction on the presence of the moiety (8) in (2a) was conclusively established by *X*-ray analysis of cyclopiamine B.

Crystal Structure of Cyclopiamine B (2a).—Crystals of cyclopiamine B (2a) were obtained from a supersaturated solution of cyclopiamine B in a 2 : 1 mixture of ethyl acetate and methanol. Light yellow prisms, ranging in length from 0.5 to 3 mm appeared during a 24 h period from the solution at room temperature.

Crystal Data.—Cyclopiamine B (2a): $C_{26}H_{33}N_3O_5 \cdot CH_3OH$, Orthorhombic, $a = 16.61(1)$, $b = 14.32(1)$, $c = 10.80(1)$ Å, $U = 2\,568.8$ Å^3, $F(000) = 1\,072$, μ(Mo-K_α) = 0.54 cm^{-1}, ρ(calc.) = 1.29 g cm^{-3}.

Preliminary oscillation and Weissenberg photographs established the space group as $P2_12_12_1$ and cell constants were refined on a Phillips PW 1100 diffractometer before data collection. Mo-K_α Radiation obtained with a graphite crystal monochromator was used and a total of 1 408 intensities were measured in ω—2θ scan mode over 0.8°, in steps of 0.025° s^{-1} within the limits $3 \leqslant \theta \leqslant 20°$. Background correction was made by counting for half the scan time at each end of the scan and subtracting the total count. After standard data reduction the structure was solved by direct methods and refined by full-matrix least squares using the program SHELX-76 for all computations. In order to locate the hydrogen atoms on a difference map, it was at first necessary to refine anisotropic thermal parameters for the oxygen atoms. Two hydrogen atoms on methyl groups which failed to show up were entered at geometrically calculated positions before refinement. The identification of C, N, and O atoms according to their isotropic vibration parameters was in line with the observed molecular geometry.*

* The structure factors for this work together with the thermal parameters are deposited as a Supplementary Publication [SUP No. 22489 (13 pp.)]. For details of this scheme, see Notice to Authors No. 7, *J.C.S. Perkin I*, 1978, Index issue.

The relative stereochemistry of cyclopiamine B is shown stereoscopically in Figure 1; the absolute configuration was not determined. The packing diagram in Figure 2 shows that the crystal contains methanol of

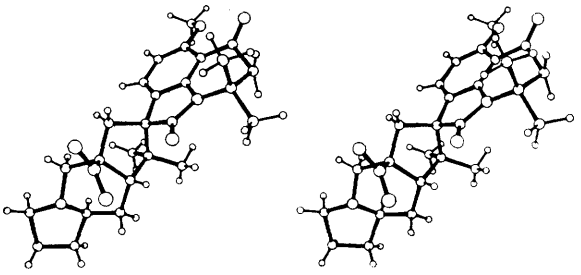


FIGURE 1 Stereoscopic drawing of cyclopiamine B

crystallization, probably hydrogen-bonded to the main molecule. The atomic co-ordinates at the termination of refinement, when $R = 0.06$, are given in Table 1 and the numbering scheme is illustrated in Figure 3. The

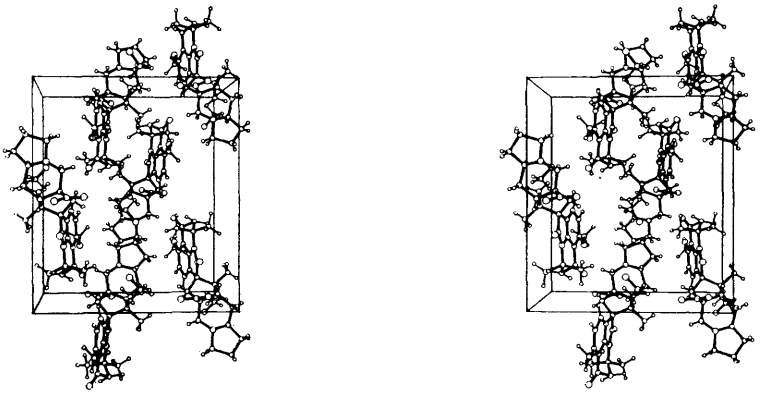


FIGURE 2 Stereoscopic packing diagram of cyclopiamine B with methanol of crystallization

numbering of the hydrogen atoms follows the numbers of non-hydrogen atoms to which they are attached.

The molecule of cyclopiamine B consists of two systems of three fused rings each, connected at a spiro-carbon atom, C(1). The two systems *viz.* A,B,C and D,E,F are virtually perpendicular to one another due to the spiro-junction. The bond lengths and angles for cyclopiamine B are given in Tables 2 and 3. The relative torsion angles are given in Table 4. The conformations of individual rings, described in terms of parameters of pucker [15] are summarised in Table 5. The conformational nomenclature used in the discussion that follows has been defined elsewhere.[16]

A noticeable feature of the molecule is the deviation from tetrahedral geometry at the spiro-centre. The bond lengths involving C(1) range from 1.50 to 1.56 Å and correspond reasonably well with known bond lengths between saturated carbon atoms at a spiro-centre.[17-19] The difference between angles C(11)–C(1)–C(2) (102°) and C(13)–C(1)–C(11) (116°) is, however, larger than any spiro-distortion observed before and may be caused by the non-bonded interaction of the C(13) methyl groups with atoms O(24) and C(11) as shown by the non-bonded contacts listed in Table 6.

Ring A is almost flat as evidenced by its amplitude of pucker, $Q = 0.12$ Å, and nearly coplanar with the aromatic ring, C, as illustrated by the exocyclic torsion angles C(1)–C(11)–C(10)–C(9) and N(3)–C(12)–C(7)–C(8) which are both equal to 178(1)°. The parameter $\phi_A = 233°$ typifies the ring as a $^2T_3$ twist form. The bond lengths of the oxindole system defined by ring A and C correlate well with those of ranboxine,[17] isoindigo,[20] and indirubine.[21]

Ring B has an envelope conformation, E_5, and the flat part is nearly coplanar with the oxindole system as defined by the torsion angles C(6)–C(7)–C(8)–C(9) = 176(1)° and C(4)–N(3)–C(12)–C(11) = 174(1)°. The geometry around C(6) deviates from the expected trigonal arrangement for an sp^2 carbon with a C(5)–C(6)–C(7) valence angle of 112° and with the oxygen atom O(27) at 0.064 Å from the plane through the carbon atoms.

The bond lengths and angles in ring D correspond well with the values for the cyclopentane ring in steroids.[22] The ring is fairly puckered and has a conformation halfway between the pseudorotational envelope and twist

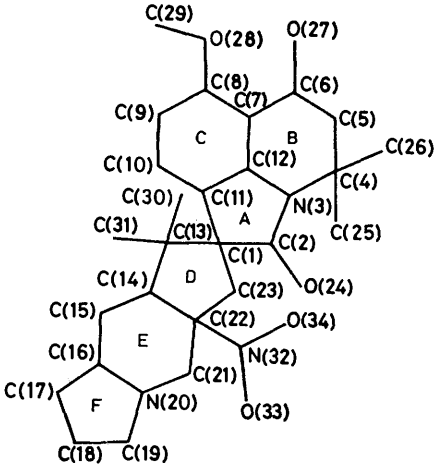


FIGURE 3 Atomic numbering scheme for the non-hydrogen atoms of cyclopiamine B

TABLE 1

Refined atomic co-ordinates ($\times 10^4$) for cyclopiamine B (2a) with e.s.d. in parentheses

Atom	x	y	z
C(1)	5 892(4)	1 237(5)	7 059(7)
C(2)	6 692(4)	1 298(5)	7 794(7)
N(3)	7 308(3)	1 386(4)	6 952(5)
C(4)	8 182(4)	1 472(5)	7 222(7)
C(5)	8 638(5)	1 277(6)	6 008(8)
C(6)	8 333(5)	1 891(5)	4 908(7)
C(7)	7 449(4)	1 808(4)	4 744(6)
C(8)	7 021(4)	1 979(5)	3 643(7)
C(9)	6 180(4)	1 931(5)	3 628(7)
C(10)	5 766(4)	1 712(5)	4 707(7)
C(11)	6 158(4)	1 520(5)	5 783(7)
C(12)	7 001(4)	1 562(5)	5 774(7)
C(13)	5 525(4)	243(5)	7 167(7)
C(14)	5 195(4)	252(5)	8 513(6)
C(15)	4 611(4)	−525(5)	8 906(7)
C(16)	3 729(5)	−298(5)	8 788(8)
C(17)	3 142(5)	−961(6)	9 414(9)
C(18)	2 444(6)	−355(6)	9 857(8)
C(19)	2 686(5)	648(6)	9 461(8)
N(20)	3 569(3)	606(4)	9 406(5)
C(21)	3 947(4)	1 345(5)	8 693(7)
C(22)	4 859(4)	1 254(5)	8 697(7)
C(23)	5 256(4)	1 870(5)	7 711(7)
O(24)	6 769(3)	1 258(4)	8 899(4)
C(25)	8 440(7)	709(9)	8 156(11)
C(26)	8 340(6)	2 446(6)	7 719(9)
O(27)	8 779(3)	2 322(5)	4 265(5)
O(28)	7 466(3)	2 157(4)	2 623(4)
C(29)	7 094(5)	2 049(6)	1 443(8)
C(30)	6 139(6)	−534(7)	7 022(10)
C(31)	4 876(6)	86(7)	6 196(10)
N(32)	5 116(4)	1 601(5)	10 004(7)
O(33)	5 266(3)	1 034(4)	10 801(5)
O(34)	5 101(4)	2 443(5)	10 171(6)
O(35)	3 660(5)	268(6)	2 116(8)
C(36)	4 242(8)	−288(9)	2 673(12)
H(5.1)	8 873(38)	1 742(45)	6 073(62)
H(5.2)	8 537(40)	519(49)	5 971(64)
H(9)	5 856(38)	2 076(45)	2 911(63)
H(10)	5 196(39)	1 845(46)	4 738(60)
H(14)	5 652(38)	201(44)	8 930(65)
H(15.1)	4 762(39)	−1 082(44)	8 494(62)
H(15.2)	4 737(41)	−658(48)	9 811(62)
H(16)	3 589(40)	−334(44)	7 883(63)
H(17.1)	3 100(37)	−1 418(45)	8 805(62)
H(17.2)	3 386(38)	−1 194(46)	10 172(61)
H(18.1)	2 157(38)	−593(46)	9 298(60)
H(18.2)	2 360(39)	−411(43)	10 701(59)
H(19.1)	2 505(39)	1 216(44)	10 057(58)
H(19.2)	2 441(40)	817(44)	8 557(59)
H(21.1)	3 805(37)	1 911(46)	8 966(63)
H(21.2)	3 757(39)	1 303(45)	7 728(64)
H(23.1)	5 524(39)	2 365(46)	8 073(61)
H(23.2)	4 903(39)	2 020(45)	7 140(60)
H(25.1)	8 376(50)	145(60)	7 550(80)
H(25.2)	8 135(47)	417(58)	8 855(80)
H(25.3)	8 483(48)	1 246(62)	8 644(79)
H(26.1)	8 211(51)	2 964(61)	7 019(83)
H(26.2)	8 971(53)	2 475(61)	7 957(84)
H(26.3)	7 982(54)	2 586(61)	8 532(83)
H(29.1)	7 560(53)	2 187(63)	771(85)
H(29.2)	6 623(52)	2 565(64)	1 352(84)
H(29.3)	6 847(54)	1 362(65)	1 286(86)
H(36.1)	4 410(105)	−830(109)	2 028(125)
H(36.2)	4 133(111)	−592(115)	3 573(131)
H(36.3)	4 722(107)	217(114)	2 740(129)

forms ^5E and $^5T_1$. The nitro-group at the D,E ring junction appears normal. The D and E rings are *cis*-fused since the torsion angles at the junction have the same sign.[23]

The E ring has a flattened-chair conformation halfway between $^4C_1$ ($\phi = 180°$) and E_1 ($\phi = 125.3°$). The N–C bonds in the ring are slightly shorter than the C–C bonds. Karle [24] found a similar result in dealing with a comparable saturated nitrogen bridge-head. The E and F rings are *trans*-fused since the torsion angles at the junction have opposite signs. The five-membered F ring has a pure E_1 envelope conformation.

Correlation of Cyclopiamine A and B.—The u.v., i.r., and mass spectral properties of cyclopiamine A (1) were

TABLE 2

Bond lengths of cyclopiamine B (2a) (Å) (with standard deviations in parentheses) involving non-hydrogen atoms

C(1)–C(2)	1.55(1)	C(11)–C(12)	1.40(1)
C(1)–C(11)	1.50(1)	C(13)–C(14)	1.55(1)
C(1)–C(13)	1.55(1)	C(13)–C(30)	1.52(1)
C(1)–C(23)	1.56(1)	C(13)–C(31)	1.52(1)
C(2)–N(3)	1.38(1)	C(14)–C(15)	1.54(1)
C(2)–O(24)	1.20(1)	C(15)–C(16)	1.51(1)
N(3)–C(4)	1.49(1)	C(16)–C(17)	1.52(1)
N(3)–C(12)	1.39(1)	C(16)–N(20)	1.48(1)
C(4)–C(5)	1.54(1)	C(17)–C(18)	1.52(1)
C(4)–C(25)	1.55(2)	C(18)–C(19)	1.55(1)
C(4)–C(26)	1.52(1)	C(19)–N(20)	1.47(1)
C(5)–C(6)	1.56(1)	N(20)–C(21)	1.45(1)
C(6)–O(7)	1.49(1)	C(21)–C(22)	1.52(1)
C(6)–O(27)	1.19(1)	C(22)–C(14)	1.55(1)
C(7)–C(8)	1.41(1)	C(22)–C(23)	1.53(1)
C(7)–C(12)	1.38(1)	C(22)–N(32)	1.56(1)
C(8)–C(9)	1.40(1)	O(28)–C(29)	1.43(1)
C(8)–O(28)	1.35(1)	N(32)–O(33)	1.21(1)
C(9)–C(10)	1.39(1)	N(32)–O(34)	1.22(1)
C(10)–C(11)	1.36(1)		

TABLE 3

Bond angles (°) of cyclopiamine B (2a) (with standard deviations in parentheses), involving non-hydrogen atoms only

C(11)–C(1)–C(2)	101.6(5)	C(12)–C(11)–C(10)	117.6(7)
C(13)–C(1)–C(2)	110.5(6)	C(7)–C(12)–N(3)	125.7(6)
C(13)–C(1)–C(11)	115.6(6)	C(11)–C(12)–N(3)	110.6(6)
C(23)–C(1)–C(2)	108.5(6)	C(11)–C(12)–C(7)	123.6(6)
C(23)–C(1)–C(11)	117.1(6)	C(14)–C(13)–C(1)	101.6(6)
C(23)–C(1)–C(13)	103.5(5)	C(30)–C(13)–C(1)	113.6(6)
N(3)–C(2)–C(1)	107.8(6)	C(31)–C(13)–C(1)	111.3(6)
O(24)–C(2)–C(1)	126.7(7)	C(31)–C(13)–C(30)	107.2(7)
O(24)–C(2)–N(3)	125.5(7)	C(15)–C(14)–C(13)	118.4(6)
C(4)–N(3)–C(2)	127.3(6)	C(22)–C(14)–C(13)	104.7(5)
C(12)–N(3)–C(2)	110.3(6)	C(22)–C(14)–C(15)	114.0(6)
C(12)–N(3)–C(4)	121.4(6)	C(16)–C(15)–C(14)	115.7(6)
C(5)–C(4)–N(3)	107.3(6)	C(17)–C(16)–C(15)	116.9(7)
C(25)–C(4)–N(3)	109.8(7)	N(20)–C(16)–C(15)	109.0(6)
C(25)–C(4)–C(5)	106.9(7)	N(20)–C(16)–C(17)	103.3(6)
C(26)–C(4)–N(3)	108.3(6)	C(18)–C(17)–C(16)	105.8(7)
C(26)–C(4)–C(5)	112.5(7)	C(19)–C(18)–C(17)	104.1(7)
C(26)–C(4)–C(25)	111.8(8)	N(20)–C(19)–C(18)	103.4(7)
C(6)–C(5)–C(4)	112.7(7)	C(19)–N(20)–C(16)	103.5(6)
C(7)–C(6)–C(5)	111.5(6)	C(21)–N(20)–C(16)	108.7(6)
O(27)–C(6)–C(5)	122.4(7)	C(21)–N(20)–C(19)	115.0(6)
O(27)–C(6)–C(7)	126.0(7)	C(22)–C(21)–N(20)	111.6(6)
C(8)–C(7)–C(6)	125.9(6)	C(21)–C(22)–C(14)	115.9(6)
C(12)–C(7)–C(6)	117.1(6)	C(23)–C(22)–C(14)	106.7(6)
C(12)–C(7)–C(8)	116.9(6)	C(23)–C(22)–C(21)	112.3(6)
C(9)–C(8)–C(7)	120.3(6)	N(32)–C(22)–C(14)	108.2(6)
O(28)–C(8)–C(7)	116.5(6)	N(32)–C(22)–C(21)	104.4(6)
O(28)–C(8)–C(9)	123.1(6)	N(32)–C(22)–C(23)	109.2(6)
C(10)–C(9)–C(8)	119.7(7)	C(22)–C(23)–C(1)	105.8(5)
C(11)–C(10)–C(9)	121.7(7)	C(29)–O(28)–C(8)	118.2(6)
C(10)–C(11)–C(1)	134.2(6)	O(33)–N(32)–C(22)	119.2(7)
C(12)–C(11)–C(1)	108.2(6)	O(34)–N(32)–C(22)	116.4(7)
		O(34)–N(32)–O(33)	124.2(8)

TABLE 4

Torsion angles of cyclopiamine B (2a) (with standard deviations in parentheses), involving non-hydrogen atoms only

	Torsion angle (°)
C(1)–C(2)–N(3)–C(4)	−179.6(7)
C(1)–C(2)–N(3)–C(12)	−10.9(13)
C(1)–C(11)–C(10)–C(9)	177.7(7)
C(1)–C(11)–C(12)–N(3)	3.6(12)
C(1)–C(11)–C(12)–C(7)	179.6(7)
C(1)–C(13)–C(14)–C(15)	−166.2(6)
C(1)–C(13)–C(14)–C(22)	−37.8(10)
C(1)–C(23)–C(22)–C(14)	6.3(11)
C(1)–C(23)–C(22)–C(21)	134.2(7)
C(1)–C(23)–C(22)–N(32)	−110.6(7)
C(2)–C(1)–C(11)–C(10)	172.2(6)
C(2)–C(1)–C(11)–C(12)	−9.4(11)
C(2)–C(1)–C(13)–C(14)	−74.4(9)
C(2)–C(1)–C(13)–C(30)	43.6(10)
C(2)–C(1)–C(13)–C(31)	164.8(6)
C(2)–C(1)–C(23)–C(22)	87.4(8)
C(2)–N(3)–C(4)–C(5)	−164.4(6)
C(2)–N(3)–C(4)–C(25)	−48.5(11)
C(2)–N(3)–C(4)–C(26)	73.6(10)
C(2)–N(3)–C(12)–C(7)	−171.0(7)
C(2)–N(3)–C(12)–C(11)	4.8(13)
N(3)–C(2)–C(1)–C(11)	12.2(11)
N(3)–C(2)–C(1)–C(13)	−110.9(7)
N(3)–C(2)–C(1)–C(23)	136.3(6)
N(3)–C(4)–C(5)–C(6)	−52.8(10)
N(3)–C(12)–C(7)–C(6)	0.0(13)
N(3)–C(12)–C(7)–C(8)	178.4(7)
N(3)–C(12)–C(11)–C(10)	−177.6(7)
C(4)–N(3)–C(2)–O(24)	1.6(13)
C(4)–N(3)–C(12)–C(7)	−1.5(13)
C(4)–N(3)–C(12)–C(11)	174.3(7)
C(4)–C(5)–C(6)–C(7)	53.4(9)
C(4)–C(5)–C(6)–O(27)	−130.3(6)
C(5)–C(4)–N(3)–C(12)	28.0(11)
C(5)–C(6)–C(7)–C(8)	156.3(6)
C(5)–C(6)–C(7)–C(12)	−25.4(11)
C(6)–C(5)–C(4)–C(25)	−170.6(6)
C(6)–C(5)–C(4)–C(26)	66.6(9)
C(6)–C(7)–C(8)–C(9)	176.0(7)
C(6)–C(7)–C(8)–O(28)	−6.1(13)
C(6)–C(7)–C(12)–C(11)	−175.3(7)
C(7)–C(8)–C(9)–C(10)	0.2(13)
C(7)–C(8)–O(28)–C(29)	−161.0(8)
C(7)–C(12)–C(11)–C(10)	−1.7(13)
C(8)–C(7)–C(6)–O(27)	−19.9(11)
C(8)–C(7)–C(12)–C(11)	3.1(13)
C(8)–C(9)–C(10)–C(11)	1.4(13)
C(9)–C(8)–C(7)–C(12)	−2.3(13)
C(9)–C(8)–O(28)–C(29)	16.8(4)
C(9)–C(10)–C(11)–C(12)	−0.7(14)
C(10)–C(9)–C(8)–O(28)	−177.6(7)
C(10)–C(11)–C(1)–C(13)	−68.3(10)
C(10)–C(11)–C(1)–C(23)	54.1(10)
C(11)–C(1)–C(2)–O(24)	−169.0(6)
C(11)–C(1)–C(13)–C(14)	171.0(6)
C(11)–C(1)–C(13)–C(30)	−71.1(9)
C(11)–C(1)–C(13)–C(31)	50.2(10)
C(11)–C(1)–C(23)–C(22)	−158.4(6)
C(12)–N(3)–C(2)–O(24)	170.3(7)
C(12)–N(3)–C(4)–C(25)	143.9(7)
C(12)–N(3)–C(4)–C(26)	−94.0(9)
C(12)–C(7)–C(6)–O(27)	158.4(6)
C(12)–C(7)–C(8)–O(28)	175.6(7)
C(12)–C(11)–C(1)–C(13)	110.2(8)
C(12)–C(11)–C(1)–C(23)	−127.4(7)
C(13)–C(1)–C(2)–O(24)	67.8(9)
C(13)–C(1)–C(23)–C(22)	−29.9(10)
C(13)–C(14)–C(15)–C(16)	94.2(8)
C(13)–C(14)–C(22)–C(21)	−106.0(7)
C(13)–C(14)–C(22)–C(23)	19.8(10)
C(13)–C(14)–C(22)–N(32)	137.2(6)
C(14)–C(13)–C(1)–C(23)	41.6(10)
C(14)–C(15)–C(16)–C(17)	167.6(6)
C(14)–C(15)–C(16)–N(20)	51.0(10)
C(14)–C(22)–C(21)–N(20)	−43.1(10)
C(14)–C(22)–N(32)–O(33)	24.3(10)
C(14)–C(22)–N(32)–O(34)	−160.5(6)
C(15)–C(14)–C(13)–C(30)	73.1(9)
C(15)–C(14)–C(13)–C(31)	−46.8(10)
C(15)–C(14)–C(22)–C(21)	25.2(10)
C(15)–C(14)–C(22)–C(32)	150.9(6)
C(15)–C(14)–C(22)–N(32)	−91.7(8)
C(15)–C(16)–C(17)–C(18)	−146.1(6)
C(15)–C(16)–N(20)–C(19)	168.4(6)
C(15)–C(16)–N(20)–C(21)	−68.8(10)
C(16)–C(15)–C(14)–C(22)	−29.7(10)
C(16)–C(17)–C(18)–C(19)	0.8(11)
C(16)–N(20)–C(19)–C(18)	−42.8(11)
C(16)–N(20)–C(21)–C(22)	65.1(10)
C(17)–C(16)–N(20)–C(19)	43.4(11)
C(17)–C(16)–N(20)–C(21)	166.2(6)
C(17)–C(18)–C(19)–N(20)	25.4(10)
C(18)–C(17)–C(16)–N(20)	−26.5(11)
C(18)–C(19)–N(20)–C(21)	−161.3(6)
C(19)–N(20)–C(21)–C(22)	−179.4(6)
N(20)–C(21)–C(22)–C(23)	−166.0(6)
N(20)–C(21)–C(22)–N(32)	75.9(9)
C(21)–C(22)–N(32)–O(33)	−99.7(8)
C(21)–C(22)–N(32)–O(34)	75.5(9)
C(22)–C(14)–C(13)–C(30)	−158.5(6)
C(22)–C(14)–C(13)–C(31)	81.6(8)
C(23)–C(1)–C(2)–O(24)	−45.0(10)
C(23)–C(1)–C(13)–C(30)	159.6(6)
C(23)–C(1)–C(13)–C(31)	−79.2(8)
C(23)–C(22)–N(32)–O(33)	140.1(6)
C(23)–C(22)–N(32)–O(34)	−44.7(10)

virtually identical to those of cyclopiamine B (2a). The pertinent differences in the ^{1}H n.m.r. spectra of the compounds were confined to protons in the D,E,F ring system which resonated in the δ 2—4 region. The close structural relationship of (1) and (2a) is based on the observation that heating of cyclopiamine A in polar solvents, *e.g.* dioxan–water, ethanol–water, or dimethylformamide, leads to the formation of an equilibrium mixture of cyclopiamine A (1) and cyclopiamine B (2a) in a ratio of 1 : 7. The same ratio was also obtained upon the heating of (2a) in these solvents but the equilibration required substantially longer reaction times. The same equilibrium mixture was also obtained upon prolonged heating of (1) in chloroform–methanol (1 : 1). This solvent mixture was employed for the extraction of the mouldered material of *P. cyclopium* and to yield (1) and (2a) in approximately equal quantities. It is thus probable that only one of the metabolites, *viz.* cyclopiamine A is a true fungal metabolite, and that cyclopiamine B is an artefact produced in the extraction process.

It is evident that the mechanism of the foregoing reaction could lead to the structure of cyclopiamine A. Cyclopiamine A was stable in non-polar solvents (*e.g.* benzene and chloroform), and this suggested that the isomerisation proceeded *via* a highly polar transition state or intermediate. Furthermore, the rate of isomerisation was not significantly altered by the addition of one equivalent of acetic acid. The addition of strong mineral acid (sulphuric or hydrochloric acid), however, prevented the isomerisation. This suggested that the basic nitrogen was involved in the isomerisation reaction. The isomerisation of cyclopiamine A in pyridine at 80 °C was slower than in dimethylformamide at the

TABLE 5

Parameters of pucker and conformation of the rings in cyclopiamine B (2a)

Ring	Amplitude Q(Å)	ϕ (°)	θ (°)	Conformation
A[N(3)–C(2)–C(1)–C(11)–C(12)]	0.12	233		$^2T_3$
B[N(3)–C(12)–C(7)–C(6)–C(5)–C(4)]	0.47	62	127	E_5
C[C(7)–C(12)–C(11)–C(10)–C(9)–C(8)]	0.03	18	74	flat
D[C(1)–C(23)–C(22)–C(14)–C(13)]	0.42	152		$^5E \leftrightarrow {}^5T_1$
E[N(20)–C(16)–C(15)–C(14)–C(22)–C(21)]	0.54	191	149	$^4C_1 \leftrightarrow E_1$
F[N(20)–C(19)–C(18)–C(17)–C(16)]	0.42	180		$_1E$

same temperature. The foregoing results indicated that the isomerisation did not require acid or base catalysis. The nitro-group must be directly involved in the isomerisation process as the primary amine, obtained by

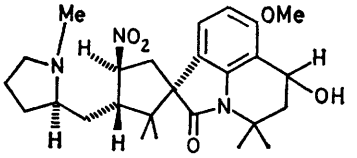
TABLE 6

Intramolecular non-bonded distances between atoms in the spiro-region of cyclopiamine B

Atoms	Distance (Å)
O(24)–H(23.1)	2.75
O(24)–H(30.2)	2.74
O(24)–H(14)	2.40
C(10)–H(31.1)	2.81
C(11)–H(30.1)	2.85
C(11)–H(31.1)	2.87
C(11)–H(31.2)	3.10
C(31)–H(10)	3.02

sodium dithionite reduction of cyclopiamine A, was completely stable upon heating in dimethylformamide or other polar solvents. The equilibration probably occurs in the β-amino-nitroalkane grouping located in the D,E,F ring system through cleavage and reformation of the C(21)–C(22) bond as shown in Scheme 2.

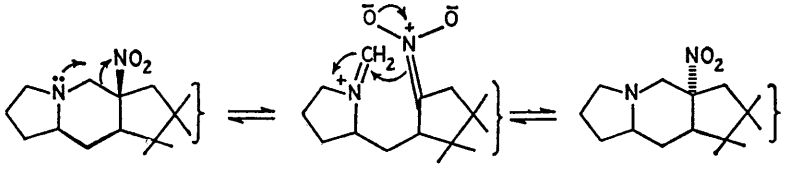
a *trans*-relationship with the alkyl substituent at C(14), thereby ensuring a minimisation of the 1,2-steric interaction between substituents. The *N*-methyl group in (11) clearly originated from the C(21) methylene group



(11)

in cyclopiamine A (1); in this compound (1) the C(21) protons appear as an AB system at δ 3.05 and 2.47 (J 15 Hz). The mass spectrum of this seco-compound (11) was dominated by the fragmentation (α-cleavage) of the carbon–carbon bond adjacent to the pyrrolidine ring ststem giving rise to the base peak at *m/e* 84 ($C_5H_{10}N^+$).

Application of the classical Nef reaction [26] to (11) furnished the main products (12) and (13). The latter



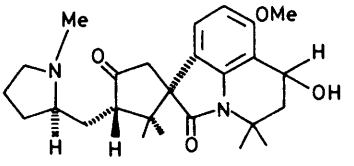
SCHEME 2

Direct evidence for the intermediacy of an immonium ion during the isomerisation was derived from heating cyclopiamine A in dioxan–water containing an excess of potassium borohydride. This resulted in the trapping of the immonium ion intermediate and the reduction of the ketone group to give the diastereoisomeric alcohols (11). The main diastereoisomer could not be induced to crystallise. Its chromatographic and spectral properties, however, established its purity. In the ^{1}H n.m.r. spectrum, a three-proton singlet at δ 2.35 was assigned to the *N*-methyl group, whereas a multiplet at δ 4.65 was assigned to the methine proton on the nitro-bearing carbon atom. The foregoing methine proton could be exchanged for deuterium by treatment of (11) with deuterium oxide and a trace of triethylamine. This proton formed the X portion of an ABMX system with $J_{BX} = 8.5$ Hz and $J_{MX} = 6.0$ Hz. The coupling of the C(23) methylene protons with H_X is in agreement with a mobile five-membered ring whereas the vicinal coupling (J_{MX} 6 Hz) is in agreement with a *trans*-relationship of the two protons.[25] The nitro-group, therefore, appears to have compound clearly originated from acid-catalysed dehydration of (12). The i.r. spectra of (12) and (13) showed strong absorption at 1 746 cm^{-1} due to the cyclopentanone carbonyl group. Treatment of (12) with deuteriomethanol containing sodium methoxide effected the exchange of three enolisable protons, α to the ketone group.

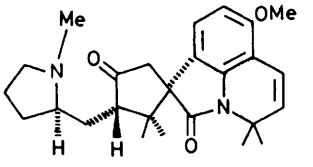
The evidence described above establishes the stereochemical relationship between cyclopiamines A and B. The greater thermodynamic stability of cyclopiamine B required further attention. The structure and relative stereochemistry of (2a) as obtained by single crystal *X*-ray analysis showed that the D,E,F tricyclic ring system has the conformation (14) (with ring E in the flattened chair form). Conformation analysis of the structure suggests that (14) represents the preferred conformation of the D,E,F ring system. However, the activation energy for nitrogen inversion is less than 10 kcal mol^{-1}.[27] In solution, (14) could therefore readily be in equilibrium with the conformation (15) in which rings E and F are *cis*-fused. In the equilibrium between the two conform-

ations, the former (14) must predominate since the *trans*-fused indolizidine ring system is known to be considerably more stable ($\Delta G^\circ = -2.4$ kcal mol^{-1}) than the corresponding *cis*-fused ring system.[28] In addition conformation (15) is also destabilised by strong 1,3-diaxial interaction [C(22)–NO_2 and C(19)–H_2].

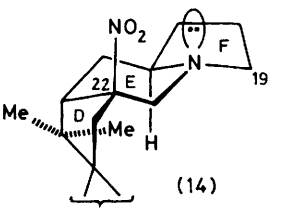
Two conformations can be considered for the D,E,F ring system of cyclopiamine A (1), *viz.* conformation (16)



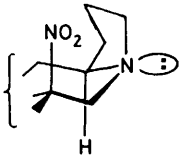
(12)



(13)

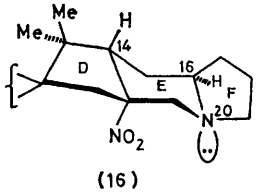


(14)

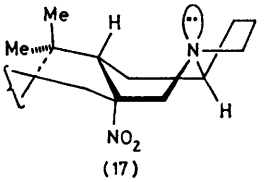


(15)

in which rings E and F are *cis*-fused, with ring E in a chair conformation, and conformation (17) in which rings E and F are *trans*-fused with ring E in a boat or twist-boat conformation. From stereochemical considerations [27,29] it was deduced that (16) represents the preferred conformation of the D,E,F ring system of cyclopiamine A.



(16)



(17)

From inspection of molecular models of the preferred conformation of cyclopiamine A and cyclopiamine B, *viz.* (16) and (14), respectively, it was clear that several factors destabilise the preferred conformation of cyclopiamine A. The main factor is that rings E and F are *trans*-fused in cyclopiamine B whereas the corresponding rings are *cis*-fused in cyclopiamine A. Direct evidence that the E,F ring systems in cyclopiamine A and B in solution are *cis*- and *trans*-fused, respectively, was obtained from the i.r. spectra of the compounds. The i.r. spectrum of cyclopiamine A shows weak absorption in the region 2 800—2 600 cm^{-1}, whereas cyclopiamine B displayed strong well-defined maxima (Bohlman bands) [20] at 2 800 and 2 750 cm^{-1}. Extensive investigations of the i.r. spectra of indolizidines and other saturated systems possessing nitrogen at the ring junction showed that strong absorption in the 2 800—2 600 cm^{-1} region is a reliable indication of *trans*-fused conformations.[30,31]

The second fused-ring system (D,E,F) present in the cyclopiamines has to our knowledge not been encountered in natural products. The cyclopiamines seem to be biogenetically derived from tryptophan, proline, and two units of dimethylallyl-pyrophosphate.

EXPERIMENTAL

M.p.s were recorded on a Kofler hot-stage apparatus. U.v. spectra were recorded with a Shimadzu UV 200 spectrophotometer in methanol (unless otherwise specified). I.r. spectra were measured with a Perkin-Elmer 257 spectrophotometer as solutions in chloroform unless otherwise stated. N.m.r. spectra were obtained with a Varian HA-100 spectrometer in deuteriochloroform with tetramethylsilane as internal reference. Mass spectra were recorded with an A.E.I. MS9 spectrometer by the direct insertion method. C.d. spectra were taken on a JASCO ORD/UV-5 spectrometer with c.d. attachment; concentration (*c*) of solution in methanol is expressed as mg ml^{-1} throughout. Solvent mixtures are given on a volume/volume basis. T.l.c. was carried out on 0.1-mm precoated Silica gel plates. *X*-Ray data were collected on the Phillips PW 1100 diffractometer at the N.P.R.L.

The Isolation of Cyclopiamine A and B.—P. cyclopium Westling (C.S.I.R. No. 1082) was grown on sterilized maize meal (15 kg) for 16 days. The fermented maize meal was dried and extracted with chloroform–methanol (1 : 1) for 2 days. The solvent was evaporated and the residue (1.3 kg) dissolved in chloroform (5 l), washed with water (3 × 2 l), and dried (Na_2SO_4). Evaporation of the chloroform fraction furnished material (700 g) which was distributed between 95% methanol (3 l) and hexane (5 l). The methanolic phase was evaporated to dryness and the residue (220 g) was dissolved in a minimum volume of chloroform. The solution was diluted with ether (3 l) and extracted with ice-cold 4M-HCl (4 × 1 l). The combined aqueous extracts were neutralized (Na_2CO_3) and extracted with chloroform (1 l). The chloroform solution was washed with water (2 × 300 ml) and dried (Na_2SO_4). Concentration of the chloroform phase furnished a thick brown oil (*ca.* 6 g) which was chromatographed over formamide-impregnated cellulose powder (3 kg). The column was developed with benzene–hexane mixtures. Elution with benzene–hexane (2 : 1) afforded a fraction (3.2 g) containing two major blue-green fluorescent compounds [R_F 0.35 and 0.55 by t.l.c. with chloroform–methanol (97 : 3) as mobile phase]. This fraction was chromatographed on silica (250 g). Elution of the column with chloroform–methanol (99 : 1) furnished

cyclopiamine B (2a) (0.95 g). After crystallisation from methanol it had m.p. 245—246 °C; $\lambda_{max.}$ 232, 260, and 345 nm (ε 16 050, 10 650, and 5 740); $\nu_{max.}$ 1 718, 1 686, 1 616, 1 549, and 1 370 cm^{-1}; Δε (*c*, 0.195) (370 nm) −0.5, (353) −2.99, (338) 0, (290) 5.98, (262) 9.59, (252) 0, (245) −19.14, (234) 0, (230) 8.38; δ 7.22 (1 H, d, *J* 8 Hz), 6.48 (1 H, d, *J* 8 Hz), 3.91 (3 H, s), 3.85 (2 H, m), 2.92 (1 H, d, *J* 15 Hz), 2.90 (1 H, m), 2.80 (1 H, d, *J* 15 Hz), 2.50 (1 H, d, *J* 15 Hz), 2.40 (1 H, d, *J* 15 Hz), 1.71 (3 H, s), 1.40 (3 H, s), 1.04 (3 H, s), and 0.90 (3 H, s); remaining protons δ 2.5 to 1.6; *m/e* (% relative intensity) 467 (16), 422 (32), 421 (100), 420 (37), 164 (11), 163 (68), 136 (28), 135 (83), 84 (14), and 70 (30) (Found: C, 66.65; H, 7.0; N, 8.8. $C_{26}H_{33}N_3O_5$ requires C, 66.79; H, 7.11; N, 8.99%).

Elution of the column with chloroform–methanol (98 : 2) gave *cyclopiamine* A (1) (1.52 g) as a colourless glass. It had: $\lambda_{max.}$ 230, 261, and 344 nm (ε 15 850, 10 300, and 5 630); $\nu_{max.}$ 1 718, 1 686, 1 616, 1 548, and 1 369 cm^{-1}; Δε (*c*, 0.163) (370 nm) −1.01, (355) −2.87, (339) 0, (301) 0.72, (295) 0, (264) 10.06, (252) 0, (242) −17.24; δ 7.23 (1 H, d, *J* 8 Hz), 6.48 (1 H, d, *J* 8 Hz), 3.88 (3 H, s), 3.05 (1 H, d, *J* 15 Hz), 2.78 (1 H, d, *J* 15 Hz), 2.48 (1 H, d, *J* 15 Hz), 2.47 (1 H, d, *J* 15 Hz), 1.73 (3 H, s), 1.41 (3 H, s), 0.96 (3 H, s), and 0.82 (3 H, s); rest of protons δ 3.2 to 1.5; *m/e* 467 (6), 422 (13), 421 (31), 420 (13), 163 (16), 135 (22), 85(70), 84 (100), 70 (12), 51 (50), 49 (100), and 47 (20) (Found: M^+, 467.242 4. $C_{26}H_{33}N_3O_5$ requires *M*, 467.242 0).

Cyclopiamine A Hydrobromide.—Cyclopiamine A (80 mg) was dissolved in 30% HBr in acetic acid (2 ml). The hydrobromide was precipitated by the addition of dry diethyl ether. The precipitate (83 mg) was collected by filtration and crystallised from methanol–acetone. *Cyclopiamine A hydrobromide* had m.p. 234—236 °C (decomp.) (Found: C, 56.85; H, 6.2; N, 7.65; Br, 14.5. $C_{26}H_{34}BrN_3O_5$ requires C, 56.91; H, 6.25; N, 7.67; Br, 14.58%).

Cyclopiamine B Hydrazone (2d).—Cyclopiamine B (100 mg) and anhydrous hydrazine (500 mg) in benzene (10 ml) were refluxed under nitrogen for 48 h. Evaporation of the solvent gave a grey powder which upon crystallisation from methanol gave *cyclopiamine B hydrazone* (2d) (62 mg), m.p. 260—261 °C (decomp.) (Found: C, 64.75; H, 7.35; N, 14.6. $C_{26}H_{35}N_5O_4$ requires C, 64.82; H, 7.33; N, 14.55%).

Monobromocyclopiamine B (2b).—Bromine (40 mg, 0.25 mmol) in acetic acid (1 ml) was slowly added to a stirred solution of cyclopiamine B (117 mg, 0.25 mmol) in acetic acid (5 ml) at 20 °C during a period of 15 min. The reaction mixture was diluted with water (50 ml) and extracted with diethyl ether (2 × 25 ml). The ether extract was washed with saturated sodium hydrogen carbonate solution (3 × 25 ml). The ether phase was dried (Na_2SO_4) and the solvent evaporated. T.l.c. [chloroform–methanol (96 : 4)] showed one major and several minor products. Chromatography on silica and elution with benzene–chloroform afforded the main product, *monobromocyclopiamine B* (2b) (75 mg). After crystallisation from ethyl acetate–benzene it had m.p. 267—268 °C (decomp.); δ 7.40 (1 H, s, aromatic proton) (Found: C, 57.15; H, 5.8; Br, 14.65; N, 7.6. $C_{26}H_{32}BrN_3O_5$ requires C, 57.12; H, 5.91; Br, 14.63; N, 7.69%).

Potassium Borohydride Reduction of Cyclopiamine A.—Potassium borohydride (53 mg, 1 mmol) in water (4 ml) (containing 1 drop of 1M-sodium hydroxide) was added dropwise to a stirred solution of cyclopiamine B (233 mg, 0.5 mmol) in methanol (40 ml) at 0 °C during a period of 15 min. The reaction mixture was kept at 0 °C for 1 h, diluted with water (100 ml), and extracted with chloroform (2 × 30 ml). This extract was dried (Na_2SO_4) and evaporated to dryness. T.l.c. [chloroform–methanol (96 : 4)] showed the presence of two products (R_F 0.58 and 0.52) in the ratio 3 : 1. Chromatography on silica (20 g) in acetone–benzene yielded fractions containing the major product (R_F 0.52, 106 mg). After crystallisation from methanol this product, *dihydrocyclopiamine B* (2e), had m.p. 266—267 °C (decomp.); $\lambda_{max.}$ 222, 263, and 288 nm (ε 24 650, 3 750, and 2 630); $\nu_{max.}$ 3 580, 3 400 (broad), 1 708, 1 633, 1 546, and 1 368 cm^{-1}; Δε (*c*, 0.202) (350 nm) 0.12, (320) 0.23, (290) 4.17, (280) 4.63, (265) 5.79, and (254) 0; δ 6.99 (1 H, d, *J* 8 Hz), 6.44 (1 H, d, *J* 8 Hz), 5.07 (1 H, t, *J* 4 Hz), 3.87 (3 H, s), 3.85 (2 H, m), 2.88 (1 H, d, *J* 15 Hz), 2.85 (1 H, m), 2.47 (2 H, m), 2.33 (1 H, d, *J* 15 Hz), 1.62 (3 H, s), 1.52 (3 H, s), 1.00 (3 H, s), and 0.81 (3 H, s) (Found: C, 66.45; H, 7.45; N, 8.75. $C_{26}H_{35}N_3O_5$ requires C, 66.50; H, 7.51; N, 8.95%).

Anhydrodihydrocyclopiamine B (3).—Phosphorus oxychloride (1 ml) was slowly added to a solution of dihydrocyclopiamine B (117 mg, 0.25 mmol) in pyridine (5 ml) at 0 °C. The mixture was heated at 80 °C for 2 h in an atmosphere of nitrogen. The reaction mixture was cooled to 0 °C, poured onto crushed ice (50 g) and extracted with chloroform. The extract was washed with 3M-HCl (4 × 30 ml) and water (30 ml). Evaporation of the organic phase afforded anhydrodihydrocyclopiamine B (3) (74 mg), m.p. 248—249 °C (from chloroform–ether); $\lambda_{max.}$ 251, 260, 280, 287, 315, and 330 nm (ε 16 150, 16 040, 8 610, 8 830, 2 800, and 1 850); $\nu_{max.}$ 1 704, 1 628, 1 552, and 1 370 cm^{-1}; δ 6.88 (1 H, d, *J* 8 Hz), 6.48 (1 H, d, *J* 10 Hz), 6.37 (1 H, d, *J* 8 Hz), 6.10 (2 H, m), 5.34 (1 H, d, *J* 10 Hz), 3.83 (3 H, s), 2.90 (1 H, d, *J* 15 Hz), 2.47 (1 H, d, *J* 15 Hz), 3.90 (1 H, m), and 1.63 (3 H, s); rest of protons δ 2.5 to 1.6 (Found: C, 69.25; H, 7.3; N, 9.2. $C_{26}H_{33}N_3O_4$ requires C, 69.16; H, 7.37; N, 9.31%).

Demethylation of Cyclopiamine B (2a).—A solution of cyclopiamine B (116 mg, 0.25 mmol) and freshly sublimed aluminium chloride (132 mg, 1 mmol) in nitrobenzene (3 ml) was stirred at room temperature for 2 h. The solution was poured onto crushed ice (20 g) and extracted with chloroform. The chloroform and nitrobenzene were removed under reduced pressure. The residue was crystallised from methanol to furnish the *phenol* (4) (81 mg), m.p. 254—256° (decomp.) (from methanol); $\lambda_{max.}$ 265 and 358 nm (ε 10 400 and 6 200); $\nu_{max.}$ 3 300—2 800, 1 718, 1 659, 1 608, 1 546, and 1 368 cm^{-1}; δ 10.58 (1 H, s, exchangeable), 7.17 (1 H, d, *J* 8 Hz), and 6.46 (1 H, d, *J* 8 Hz) (Found: C, 66.4; H, 6.9; N, 9.35. $C_{25}H_{31}N_3O_5$ requires C, 66.21; H, 6.89; N, 9.27%).

Demethylation of cyclopiamine B was also effected by heating a solution of the compound in 6M-HCl for 5 h. The phenol (4) was obtained in a yield of 73%.

Reduction of Cyclopiamine A (1) *and B* (2a) *with Sodium Dithionite*.—Sodium dithionite (348 mg, 2 mmol) was added in small portions to a stirred solution of cyclopiamine B (233 mg, 0.5 mmol) in methanol–water (1 : 4) (20 ml) at 65 °C during a period of 1 h. The mixture was then heated at 65 °C for 1 h, allowed to cool to room temperature, diluted with water (40 ml), neutralised with sodium hydrogen carbonate, and extracted with chloroform. Evaporation of the solvent gave the *primary amine* (5a) (166 mg), m.p. 275—277 °C (from chloroform); $\lambda_{max.}$ 233, 262, and 346 nm (ε 16 200, 10 700, and 5 800); $\nu_{max.}$ 3 460, 3 230 (broad), 1 716, 1 685, and 1 616 cm^{-1} (Found: C,

71.15; H, 8.1; N, 9.7. $C_{26}H_{35}N_3O_3$ requires C, 71.37; H, 8.06; N, 9.60%).

Treatment of (5a) with acetic anhydride–pyridine (1 : 1) at 20 °C for 1 h furnished the N-*acetyl derivative* (5) in quantitative yield. It had m.p. 185—187 °C (from chloroform–diethyl ether); δ 7.28 (1 H, d, *J* 8 Hz), 6.48 (1 H, d, *J* 8 Hz), 3.88 (3 H, s), 2.78 (1 H, d, *J* 15 Hz), 2.48 (1 H, d, *J* 15 Hz), 1.70 (3 H, s), 1.43 (3 H, s), 1.05 (3 H, s), and 0.87 (3 H, s); rest of protons δ 3.6 to 1.7 (Found: C, 70.25; H, 7.7; N, 8.75. $C_{28}H_{37}N_3O_4$ requires C, 70.12; H, 7.78; N, 8.77%).

Cyclopiamine A was reduced with sodium dithionite under similar conditions to those used for the reduction of cyclopiamine B. The reaction mixture was separated by chromatography on silica gel. Elution with chloroform–methanol (93 : 7) gave a *primary amine* in 87% yield. It had m.p. 189—191 °C (from methanol–diethyl ether) (Found: C, 71.2; H, 7.9; N, 9.5. $C_{26}H_{35}N_3O_3$ requires C, 71.37; H, 8.06; N, 9.60%).

Further Reduction of the Amine (5a).—The amine (5a) (218 mg, 0.5 mmol) in methanol (40 ml) containing 1 drop of 1M-sodium hydroxide, was treated at 20 °C with potassium borohydride (53 mg, 1 mmol) for 2 h. Dilution with water and extraction with chloroform gave a mixture [t.l.c., chloroform–methanol (85 : 15); R_F 0.52 and 0.47] of diastereoisomeric alcohols (7a and b) (210 mg; M^+, 439). This mixture was suspended in dry tetrahydrofuran (20 ml) and treated with lithium aluminium hydride (16 mg, 0.33 mmol). The mixture was refluxed for 3 h under dry nitrogen. The excess of reagent was destroyed by addition of ethyl acetate. After the addition of water (20 ml) and filtration, the mixture was extracted with chloroform (30 ml). The extract was washed with water and dried (Na_2SO_4). The residue (194 mg) was chromatographed on neutral alumina (20 g). Elution with chloroform–methanol (99 : 1) yielded the *carbinolamine* (7a) (103 mg). After crystallisation from chloroform–ether it had m.p. 183—185 °C; $\lambda_{max.}$ 288sh, 255, and 310 nm (ε 16 800, 7 800, and 5 450); $\lambda_{max.}$ (in 1M-HCl–CH_3OH) 221, 255, and 297 nm (ε 14 100, 11 100, and 4 450); $\nu_{max.}$ 3 550, 3 460, 3 265 (broad), and 1 626 cm^{-1}; δ 6.75 (1 H, d, *J* 8 Hz), 6.10 (1 H, d, *J* 8 Hz), 5.20 (1 H, s), 4.90 (1 H, t, *J* 4 Hz), 3.80 (3 H, s), 1.46 (3 H, s), 1.23 (6 H, s), and 0.95 (3 H, s); rest of protons δ 3.7 to 1.5 (Found: C, 70.65; H, 8.8; N, 9.45. $C_{26}H_{39}N_3O_3$ requires C, 70.71; H, 8.90; N, 9.52%).

Treatment of (7a) (0.1 mmol) in acetone (10 ml) with 4 equivalents of 0.4M-chromic acid in 4.5M-H_2SO_4 at 0 °C for 5 min gave the amine (5a) (73%), identified by its m.p. 276 °C (from chloroform), mixed m.p. and its i.r. spectrum.

The carbinolamine (7a) (95 mg) in 3M-HCl (8 ml) was kept at room temperature for 15 min. The solution was neutralised with sodium hydrogen carbonate and extracted with chloroform. Evaporation of the solvent furnished the *carbinolamine* (7b) (58 mg), m.p. 132—133 °C (from ether–benzene); $\lambda_{max.}$ 240, 250, 278, and 308 nm (ε 15 600, 7 650, 7 750, and 5 380); δ 6.78 (1 H, d, *J* 8 Hz), 6.04 (1 H, d, *J* 8 Hz), 5.60 (1 H, s), 5.04 (1 H, t, *J* 4 Hz), 3.80 (3 H, s), 1.38 (3 H, s), 1.36 (6 H, s), and 1.20 (3 H, s); rest of protons δ 3.7 to 1.5 (Found: C, 70.85; H, 9.2; N, 9.4. $C_{26}H_{39}N_3O_3$ requires C, 70.71; H, 8.85; N, 9.52%).

The Isomerization of Cyclopiamine A (1) *to Cyclopiamine B* (2b).—Cyclopiamine A (100 mg) in dioxan–water (1 : 1) was refluxed under nitrogen. The reaction was followed by t.l.c. [chloroform–methanol (97 : 3)] until no further change could be detected. The isomerisation reached equilibrium after 3 h. The products consisted of a mixture of cyclopiamine A and B (R_F 0.35 and 0.55, respectively). Chromatography on silica (10 g) and elution with chloroform–methanol (99 : 1) furnished cyclopiamine B (84 mg). Elution of the column with chloroform–methanol (98 : 2) furnished cyclopiamine A (13 mg). The products were identified by comparison of their spectral data. Essentially the same equilibrium mixture was obtained by heating cyclopiamine A with dimethylformamide (80 °C, 4 h), ethanol–water (1 : 1) (reflux 2.5 h), and chloroform–methanol (1 : 1) (reflux, 96 h), or by heating cyclopiamine B with dimethylformamide (80 °C, 12 h) or dioxan–water (1 : 1) (reflux, 9.5 h).

Reductive Cleavage of Ring E *of Cyclopiamine A* (1).—Potassium borohydride (324 mg, 6 mmol) was added in small portions during a period of 3 h to a solution of cyclopiamine A (467 mg, 1 mmol) in dioxan–water (1 : 1) (25 ml) under reflux. The reaction mixture was allowed to cool, diluted with water (50 ml), and extracted with chloroform (2 × 20 ml). T.l.c. [chloroform–methanol (5 : 1)] of the extract showed the presence of two compounds (R_F 0.42 and 0.36) in the ratio of 1 : 3. Chromatography of the total product on silica and elution with chloroform–methanol (10 : 1) led to the isolation of the major product, *viz.* the *seco-compound* (55 mg, R_F 0.36), as a colourless glass. It had $\lambda_{max.}$ 222, 263sh, and 286 nm (ε 23 100, 3 720, and 2 540 nm); $\nu_{max.}$ 1 708, 1 615, 1 526, and 1 369 cm^{-1}; δ 6.98 (1 H, d, *J* 8 Hz), 6.45 (1 H, d, *J* 8 Hz), 5.05 (1 H, t, *J* 4 Hz), 4.65 (1 H, sextet, exchangeable part of ABCX system, $J_{AX} = J_{BX}$ 8.5 Hz, J_{CX} 6.0 Hz), 3.85 (3 H, s), 2.35 (3 H, s), 1.62 (3 H, s), 1.57 (3 H, s), 0.96 (3 H, s), and 0.84 (3 H, s); rest of protons δ 3.7—1.5 (Found: C, 66.35; H, 7.8; N, 8.75. $C_{26}H_{37}N_3O_5$ requires C, 66.22; H, 7.91; N, 8.91%).

Nef Reaction of the Seco-compound (11).—The seco-compound (11) (210 mg) was dissolved in 2M-potassium hydroxide in methanol (25 ml). The yellow solution was added dropwise during a period of 30 min to 4.5M-H_2SO_4 (75 ml) at room temperature and the mixture was then stirred for a further period of 30 min; it was then diluted with water (100 ml), neutralised with sodium carbonate, and extracted with chloroform. The chloroform was removed under reduced pressure. T.l.c. [chloroform–methanol (8 : 1)] of the residue (178 mg) showed the presence of two major compounds (R_F 0.48 and 0.35) which were isolated by chromatography on silica (20 g). Elution with chloroform–methanol (97 : 3) furnished the *cyclopentanone* (13) (87 mg). It had m.p. 99—101 °C (from ether–n-hexane); $\lambda_{max.}$ 249, 262, 281, 288, and 328 nm (ε 16 300, 15 800, 8 800, and 8 950); $\nu_{max.}$ 1 748, 1 706, 1 659, 1 615, and 1 600 cm^{-1}; δ 6.91 (1 H, d, *J* 8 Hz), 6.50 (1 H, d, *J* 11 Hz), 6.42 (1 H, d, *J* 8 Hz), 5.37 (1 H, d, *J* 11 Hz), 3.84 (3 H, s), 2.36 (3 H, s), 1.67 (6 H, s), 1.10 (3 H, s), and 1.02 (3 H, s); rest of protons δ 3.7 and 1.9 (Found: C, 74.1; H, 8.25; N, 6.45. $C_{26}H_{34}N_2O_3$ requires C, 73.90; H, 8.11; N, 6.63%).

Elution of the column with chloroform–methanol (95 : 5) gave the *keto-alcohol* (12) (56 mg) as a colourless oil. It had $\lambda_{max.}$ 221, 264, and 287 nm (ε 22 800, 3 686, and 2 590); $\nu_{max.}$ 1 745, 1 708, 1 654, 1 620, and 1 600 cm^{-1} (Found: M^+, 440.285 6. $C_{26}H_{36}N_2O_4$ requires *M*, 440.285 4).

t-Butyl 1-(6-*Methoxyindol*-1-*yl*)-3,3-*dimethylpropionate* (9).—6-Methoxyindole [13] (500 mg, 3.4 mmol) in t-butyl 3-methylcrotonate (2.5 g) and dichloromethane (2 ml) was treated with potassium t-butoxide (0.38 g, 3.4 mmol) and the mixture heated at 80 °C for 20 h under nitrogen. The

mixture was diluted with water (4 ml) and extracted with chloroform. The chloroform phase was dried (Na_2SO_4). Chromatography of the residue on alumina and elution with benzene–hexane (1 : 4) yielded the N-*alkylated indole* (9) as a colourless oil (0.57 g, 55%), $\nu_{max.}$ 1 730 cm^{-1}; δ 7.48 (1 H, d, *J* 8 Hz), 7.40 (1 H, d, *J* 2 Hz), 7.20 (1 H, d, *J* 3 Hz), 6.78 (1 H, dd, *J* 8 and 2 Hz), 6.36 (1 H, d, *J* 3 Hz), 3.90 (3 H, s), 2.98 (2 H, s), 1.86 (6 H, s), and 1.22 (9 H, s) (Found: M^+, 303. $C_{18}H_{25}NO_3$ requires *M*, 303).

t-Butyl 1-(6-*Methoxyoxindol*-1-*yl*)-3,3-*dimethylpropionate* (10a).—The alkylated indole (9) (900 mg, 2.96 mmol) in 95% t-butyl alcohol was treated with *N*-bromosuccinimide (800 mg, 4.74 mmol) during a period of 15 min at 20 °C. The mixture was then stirred at 20 °C for 16 h. Sodium acetate (485 mg, 5.92 mmol), palladium–charcoal (600 mg), and ethanol (25 ml) were added to the crude mixture which was hydrogenated under atmospheric pressure at 20 °C. The catalyst and insoluble salts were removed by filtration. The solvent was evaporated off and the residue chromatographed on silica gel with benzene–ethyl acetate (9 : 1) as eluant to yield the *oxindole* (10a) (500 mg, 53%) as a colourless oil, $\nu_{max.}$ 1 740 and 1 660 cm^{-1}; δ 7.08 (1 H, d, *J* 8 Hz), 6.88 (1 H, d, *J* 2 Hz), 6.50 (1 H, dd, *J* 8 and 2 Hz), 3.80 (3 H, s), 3.36 (2 H, s), 3.00 (2 H, s), 1.96 (6 H, s), and 1.28 (9 H, s) (Found: M^+, 319. $C_{18}H_{25}NO_4$ requires *M*, 319).

1-(6-*Methoxyoxindol*-1-*yl*)-3,3-*Dimethylpropionic Acid* (10b).—The oxindole ester (10a) (300 mg, 0.94 mmol) was treated with trifluoroacetic acid (114 mg, 0.94 mmol) in chloroform (5 ml) at 20 °C for 4 h. The reaction mixture was diluted with water (20 ml) and extracted with chloroform. The organic residue was chromatographed on silica (30 g). Elution with benzene–ethyl acetate (1 : 1) afforded the *acid* (10b) (137 mg), m.p. 135—136 °C (from benzene–chloroform) (Found: C, 63.7; H, 6.45; N, 5.2. $C_{14}H_{17}NO_4$ requires C, 63.87; H, 6.51; N, 5.23%).

Ring Closure of Compound (10b).—The oxindole acid (10b) (100 mg, 0.38 mmol) in benzene (2 ml) was treated in benzene with thionyl chloride (33 μl, 0.46 mmol) (freshly distilled from quinoline) and sodium carbonate (48 mg, 0.46 mmol). The mixture was stirred at 20 °C for 3 h. The benzene and excess of thionyl chloride were removed at 20 °C under reduced pressure. The residue was dissolved in dry dichloromethane (4 ml) and heated with aluminium chloride (120 mg) at 40 °C for 30 min. Iced 5M-HCl (4 ml) and benzene (10 ml) were added. The solution was stirred for 20 min. The benzene phase was separated and the aqueous solution washed with benzene (2 × 20 ml). The benzene extract was washed with 2M-sodium hydroxide (2 × 10 ml) and then with water (2 × 10 ml). Chromatography of the organic residue on silica with benzene–ethyl acetate (4 : 1) gave (8) (9 mg, 10%), m.p. 182—183 °C (from benzene–ethyl acetate); $\lambda_{max.}$ 232, 260, and 345 nm (ε 15 900, 10 260, and 5 400); $\nu_{max.}$ 1 718 and 1 686 cm^{-1}; δ 7.13 (1 H, d, *J* 8 Hz), 6.45 (1 H, d, *J* 8 Hz), 3.94 (3 H, s), 3.56 (2 H, s), 3.24 (2 H, s), and 1.75 (6 H, s) (Found: C, 68.4; H, 6.1; N, 5.6. $C_{14}H_{15}NO_3$ requires C, 68.56; H, 6.16; N, 5.71%).

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