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CATHARANTHUS ALKALOIDS—XXXIX

TRICHOPHYLLINE—A NOVEL *ASPIDOSPERMA* ALKALOID FROM *CATHARANTHUS TRICHOPHYLLUS* (APOCYNACEAE)[1]

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Abstract—From an extract of the roots of *Catharanthus trichophyllus* (Apocynaceae) an alkaloid, trichophylline (**2**) has been isolated which is a new skeletal variation of the *Aspidosperma* framework. The structure was solved by single crystal X-ray crystallography. Sodium borohydride reduction afforded a novel cyclized product **3**.

The genus *Catharanthus* continues to be a source of new monomeric[3] and bisindole[4-6] alkaloids. Previous work from these laboratories on the Madagascan plant *Catharanthus trichophyllus* (Baker) Pichon (Apocynaceae) has afforded a number of known indole alkaloids,[7-10] as well as the new β-anilinoacrylate alkaloid cathaphylline.[9] The compound principally responsible for the *in vivo* antineoplastic activity displayed by the roots of *C. trichophyllus* is vincaleukoblastine (vinblastine, VLB).[10] Further fractionation of the root fractions of this plant which displayed *in vivo* and/or *in vitro* activity[11] led to the isolation of a new cytotoxic compound, to which we have given the name trichophylline.

Trichophylline crystallized from MeOH and in the UV spectrum displayed a similarity to the β-anilinoacrylate system. A number of factors confirmed that indeed this unit was present, including the observation of characteristic bands at 1670 and 1600 cm^{-1}, a methoxy CO group at δ 3.782 and a H-bonded NH at δ 10.644.[12] The mass spectrum, displaying a molecular ion at m/z 366, analyzing for $C_{21}H_{22}N_2O_4$, showed a base peak at m/z 214, similar to that commonly observed in tabersonine (**1**) and related alkaloids.[12]

However, the extended λ_{max} at 362 nm was about 30 nm more than that typically observed for compounds in this series,[12] suggesting the presence of an additional conjugating unit or stereo-electronic factors which would increase orbital overlap. A characteristic 14, 15- *cis* double bond was deduced from the ^{1}H NMR spectrum, one proton of which should be allylically coupled to a methylene group. Four adjacent aromatic protons were observed in the region δ 6.8–7.2 and were readily assigned based on prior literature data and coupling constants. A singlet Me group was observed at δ 1.330 together with a pair of geminal protons (J = 15.5 Hz) at δ 2.455 and δ 1.911 the former of which was further coupled (J = 1 Hz). A proton at δ 9.562 was indicative of an aldehyde group leaving a pair of methylene protons, one of which (δ 3.289) was a doublet of doublets (J = 6.5, 15.6 Hz), whereas the other (δ 4.814) was a doublet of triplets (J = 2.1, 16.1 Hz). The aliphatic region of the ^{1}H NMR spectrum of trichophylline is shown in Fig. 1.

Single crystal X-ray analysis indcated trichophylline to possess the structure **2** and the corresponding stereo structure is shown in Fig. 2. Atomic co-ordinates are listed in Table 1 and bond lengths and torsion angles in Tables 2 and 3, respectively.

The indole 5-membered ring is planar to within $\pm$0.01 Å and the γ-lactam ring to within $\pm$0.06 Å. However the amide system deviates slightly from planarity; the angle between the C-3, C-5, C-21 and N-4, C-7, 0-27 planes being 11.0°. The N atom N-4 is displaced by 0.041(3) Å from the former plane and the carbonyl C atom C-21 by 0.029(3) Å from the latter plane. These out-of-plane distortions of the amide group can be partitioned between twisting around the C–N bond and out-of-plane bending at N and the carbonyl C.[13] The parameters for this effect are χ_N 5.7°, χ_C −4.3° and τ 169.8° (i.e. a twist of 10.2°).

As expected, an intramolecular H-bond linking the indole and methoxy CO groups was also observed in the solid state. The dimensions are N . . . . O 2.63, N–H 1.03, H . . . , O 1.93 Å, N–H . . . . O 123°.

Sodium borohydride reduction of trichophylline (**2**) afforded a product displaying a molecular ion at

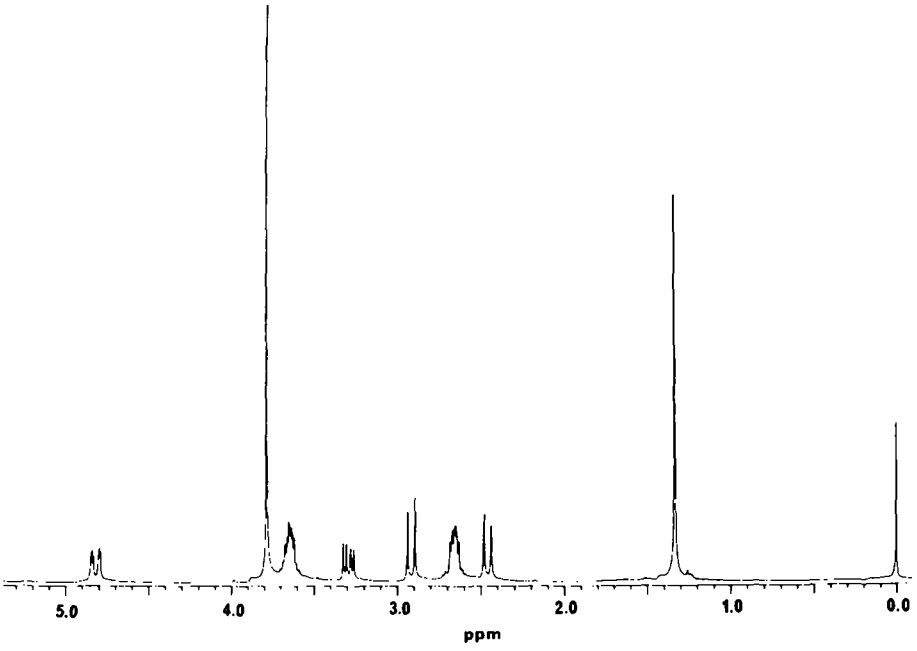


Fig. 1. Aliphatic region in the ^{1}H NMR spectrum of trichophylline (**2**).

m/z 336, 30 a.m.u. less than that of **2**. The UV spectrum of the product was essentially unchanged from that of **2** indicating that the β-anilinoacrylate unit had not been reduced. This was confirmed by the ^{1}H NMR spectrum (Fig. 3, aliphatic region only) which displayed a H-bonded NH at δ 10.449, quite similar to that observed in **2**. A number of other similarities were also observed including the 5- and 6-methylene groups at δ 3.60 and 2.83, respectively and the *cis*-14, 15-double bond at δ 5.799 and 5.902. The aldehyde group of **2** and the methoxy of the methoxy CO group were not observed in the spectrum of the reduction product suggesting that reduction of the aldehyde had occurred followed by lactonization to afford a product having the structure **3**. Such a compound would account for the upfield shift of the quaternary Me group at C-20 from δ 1.330 to δ 1.162, and the appearance of a new pair of doublets at δ 4.031 and 4.232. This relationship was confirmed through decoupling experiments. The sig-

Table 1. Atomic coordinates of trichophylline (**2**)[a]

	x	y	z
N(1)	-0.9199(4)	-0.4194(2)	-0.1666(1)
C(2)	-0.7832(4)	-0.3762(2)	-0.2096(2)
C(3)	-0.4230(6)	-0.1141(2)	-0.2420(2)
N(4)	-0.4554(4)	-0.2020(2)	-0.2046(1)
C(5)	-0.2992(5)	-0.2737(2)	-0.2022(2)
C(6)	-0.4055(5)	-0.3574(2)	-0.1700(2)
C(7)	-0.6290(4)	-0.3295(2)	-0.1599(2)
C(8)	-0.7102(5)	-0.3538(2)	-0.0872(2)
C(9)	-0.6361(5)	-0.3332(3)	-0.0205(2)
C(10)	-0.7381(6)	-0.3669(3)	0.0390(2)
C(11)	-0.9095(6)	-0.4197(3)	0.0310(2)
C(12)	-0.9824(5)	-0.4433(2)	-0.0370(2)
C(13)	-0.8787(5)	-0.4071(2)	-0.0946(2)
C(14)	-0.3683(5)	-0.1296(2)	-0.3200(2)
C(15)	-0.4628(5)	-0.1795(2)	-0.3669(2)
C(16)	-0.7914(4)	-0.3741(2)	-0.2821(2)
C(17)	-0.6271(5)	-0.3302(2)	-0.3284(2)
C(18)	-0.7396(7)	-0.2459(3)	-0.4399(2)
C(19)	-0.8212(5)	-0.1754(2)	-0.3282(2)
C(20)	-0.6615(5)	-0.2323(2)	-0.3625(2)
C(21)	-0.6324(5)	-0.2255(2)	-0.1760(2)
C(22)	-0.9606(5)	-0.4210(2)	-0.3171(2)
C(23)	-1.1092(6)	-0.4655(3)	-0.4259(2)
O(24)	-1.1050(3)	-0.4532(2)	-0.2873(1)
O(25)	-0.9424(4)	-0.4228(2)	-0.3880(1)
O(26)	-0.8413(4)	-0.0939(2)	-0.3370(2)
O(27)	-0.7763(4)	-0.1759(2)	-0.1680(1)

[a]Estimated standard deviations are in parentheses.

Table 2. Bond lengths (Å) of trichophylline (2)

N(1)-C(2)	1.367(4)	C(11)-C(12)	1.408(5)
N(1)-C(13)	1.392(5)	C(12)-C(13)	1.388(5)
C(2)-C(7)	1.543(4)	C(14)-C(3)	1.527(6)
C(2)-C(16)	1.364(5)	C(15)-C(14)	1.300(5)
C(3)-N(4)	1.466(5)	C(16)-C(17)	1.535(5)
N(4)-C(5)	1.468(5)	C(16)-C(22)	1.469(5)
N(4)-C(21)	1.339(4)	C(17)-C(20)	1.568(5)
C(5)-C(6)	1.525(5)	C(19)-O(26)	1.195(5)
C(6)-C(7)	1.553(5)	C(20)-C(15)	1.529(5)
C(7)-C(8)	1.510(5)	C(20)-C(18)	1.556(5)
C(7)-C(21)	1.532(4)	C(20)-C(19)	1.490(5)
C(8)-C(9)	1.380(5)	C(21)-O(27)	1.205(4)
C(8)-C(13)	1.367(5)	C(22)-O(24)	1.205(4)
C(9)-C(10)	1.394(5)	C(22)-O(25)	1.339(4)
C(10)-C(11)	1.380(6)	C(23)-O(25)	1.456(5)

Table 3. Torsion angles (deg) of trichophylline (2)

N(1)-C(2)-C(16)-C(17)	-175.9(3)	C(16)-C(7)-C(21)-N(4)	10.9(4)
N(1)-C(2)-C(16)-C(22)	0.5(5)	C(6)-C(7)-C(21)-O(27)	-173.2(3)
C(2)-C(16)-C(17)-C(20)	-102.8(4)	C(7)-C(2)-C(16)-C(17)	6.6(5)
C(2)-C(16)-C(22)-O(25)	-173.5(3)	C(7)-C(2)-C(16)-C(22)	-177.1(3)
C(3)-N(4)-C(5)-C(6)	-170.7(3)	C(14)-C(3)-N(4)-C(5)	56.7(4)
C(3)-N(4)-C(21)-C(7)	164.8(3)	C(14)-C(3)-N(4)-C(21)	-117.3(4)
C(3)-N(4)-C(21)-O(27)	-10.9(5)	C(15)-C(14)-C(3)-N(4)	51.8(5)
N(4)-C(5)-C(6)-C(7)	3.6(4)	C(15)-C(20)-C(19)-O(26)	38.0(5)
C(5)-N(4)-C(21)-C(7)	-9.5(4)	C(16)-C(17)-C(20)-C(15)	146.8(3)
C(5)-N(4)-C(21)-O(27)	174.8(3)	C(16)-C(17)-C(20)-C(18)	-95.7(4)
C(5)-C(6)-C(7)-C(2)	109.4(3)	C(16)-C(17)-C(20)-C(19)	19.8(4)
C(5)-C(6)-C(7)-C(8)	-132.5(3)	C(16)-C(22)-O(25)-C(23)	-177.9(3)
C(5)-C(6)-C(7)-C(21)	-8.6(4)	C(17)-C(20)-C(15)-C(14)	-83.9(5)
C(6)-C(7)-C(2)-N(1)	122.9(3)	C(17)-C(20)-C(19)-O(26)	164.3(4)
C(6)-C(7)-C(2)-C(16)	-59.2(4)	C(20)-C(15)-C(14)-C(3)	5.0(7)

nal at δ 4.232 was actually a doublet of doublets showing coupling of 10.9 Hz with the geminal proton and a further coupling of 2.8 Hz. Irradiation at δ 4.232 collapsed not only the geminal proton, but also eliminated a small coupling constant in the doublet of doublets ($J = 2.8$, 14.5 Hz) at δ 2.796. Back irradiation confirmed this relationship and also collapsed the doublet at δ 2.323 to a singlet. In the spectrum of **2**, these latter signals had been assigned to the C-17 methylene protons. Clearly in **3** a new, long-range coupling exists. A Dreiding model suggests that when the lactone ring adopts a chair conformation the 17α- and 19α- protons have a classic *W* relationship, which, in a rigid ring system, permits maximum coupling. The multiplicity of H-15 is of interest because it is deceptively simple.

Therefore the structure **3** is proposed for the reduction product of trichophylline (**2**). Molecular models indicate that this is a very rigid structure with little conformational mobility.

A number of features of the proton NMR spectra of **2** and **3** can now be discussed in more detail. The aldehyde proton in **2** was clearly observed as a doublet ($J = 1.5$ Hz) and irradiation established this to be due to a long-range effect with H-14. From its intensity with respect to the methoxy CO singlet the C-20 Me group was also coupled and this was established to be with one of the C-17 protons. Dreiding models suggest that this is probably with H-17α, which can be assigned on this basis. In **3**, with the lactone in a chair conformation, no such relationship exists for the Me group which is observed as a sharp singlet. Instead H-17α coupled to H-19α as discussed previously.

The two protons attached to C-3 undergo dramatic shifts on formation of the lactone. One is shifted upfield from δ 4.814 and the other downfield from δ 3.289. Coincidentally, these opposite and almost equal shifts cause the protons to have very similar chemical shifts in **3** at about δ 4.05. A plausible explanation of the upfield shift is that in **2**, the 3α-H lies in the plane of, and proximate to, the amide CO,

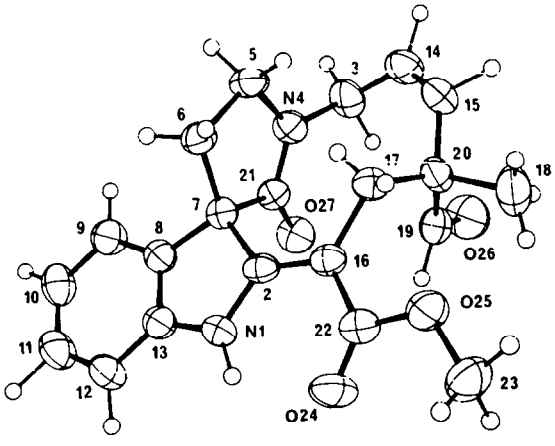


Fig. 2. ORTEP display of the structure and conformation of trichophylline (2).

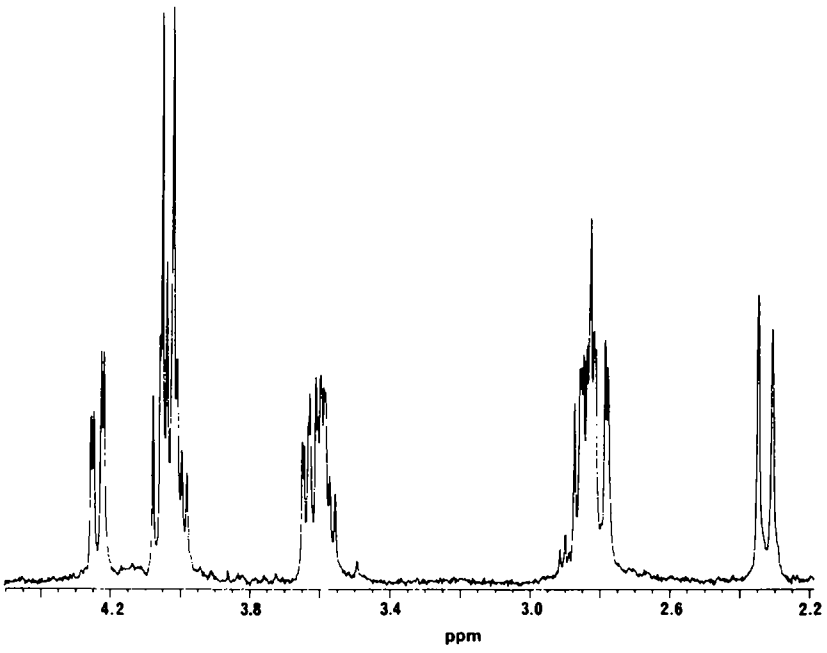
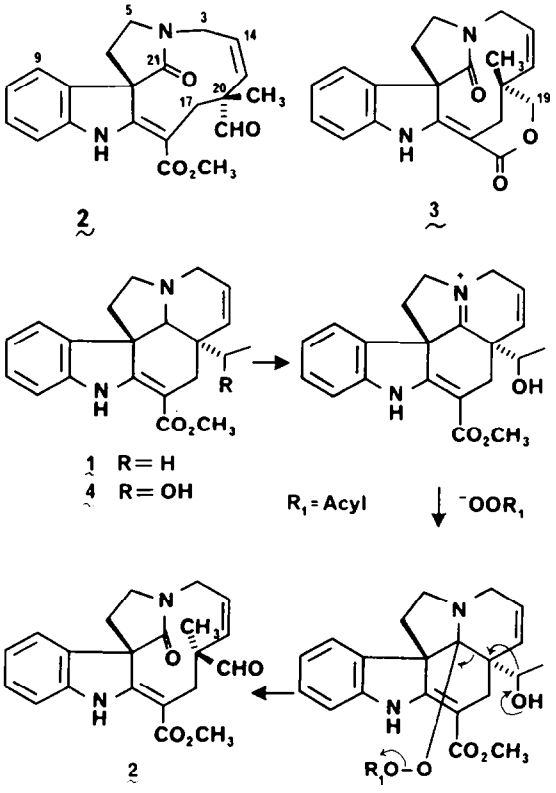


Fig. 3. Aliphatic region (2.2–4.4 ppm) in the ^{1}H NMR spectrum of the sodium borohydride reduction product of trichophylline (**2**).

whereas the 3β-H lies perpendicular to the amide and almost coplanar with the 14, 15-double bond. However, in **3** it is the 3β-H which lies in the plane of, but on the opposite side of the molecule to, the CO group, and the 3α-H which is coplanar with the 14, 15-double bond. In the former case therefore the classic shielding of a proton coplanar with an amide CO occurs.



Trichophylline (**2**) possesses two novel features for what is clearly an *Aspidosperma* alkaloid.[12] The first of these is the cleavage of the C-20-C-21 bond, and the second is the apparent migration of C-18 to C-20, or alternately a loss of C-18 with reinsertion of a Me group at C-20.

One inference of these possibilities concerns the relative stereochemistry of C-19 and C-6. The X-ray analysis of trichophylline establishes these groups to be on the same side of the molecule, unlike that in other *Aspidosperma* alkaloids.[12] This suggests that if C-18 has migrated it has occurred with inversion of configuration at C-20.

One plausible scheme for this rearrangement-fragmentation reaction is shown in Scheme 1 in which minovincinine (**4**) is oxidized at N-4-C-21. Attack of peroxide followed by deprotonation of the C-19 OH group, migration of the Me group and elimination of a carboxylate anion from an acyl peroxide at C-21 would lead to **2**.

EXPERIMENTAL

Preparation of alkaloid fractions. The source, identification and processing of the roots of *Catharanthus trichophyllus* (Baker) Pichon used in this study have been described previously.[10] Using the same procedure, *C. trichophyllus* roots (22.5 kg) afforded fraction I (110.0g), fraction II (11.4 g) and fraction III (50.0 g).

Isolation of trichophylline (**2**) *and lochnerine* (**5**). Fraction III (50.0 g) was chromatographed over a column containing silica gel (1.2 Kg) packed in $CHCl_3$. A total of 37 fractions were collected from the column and combined on the basis of the TLC pattern. Fraction 7 (2.65 g) from the column was evaporated *in vacuo* and rechromatographed over a column of silica gel (120 g). Fractions 7–8 from the column were evaporated *in vacuo*. After trituration with a little MeOH the fraction was kept refrigerated for two weeks to afford colorless crystals of **2**, (51.5 m.g. 0.00023%), m.p. 230°; $[\alpha]^{26}$ (nm) – 209.5° (589), – 220.4° (578), – 264.8° (546),

$-665.5°$ (436), -667.5 (365; UV, λ_{max} (EtOH) 238 (log ϵ 4.10), 251 (sh, 3.46), 306 (4.10), 362 (4.13) nm; IR, ν_{max} (KBr) 3250, 2900, 2820, 1721, 1713, 1687, 1658, 1651, 1600, 1574, 1435, 1200, 1195, 1187, 1112, 720 cm^{-1}; ^1H NMR, δ (360 MHz, $CDCl_3$) 1.330 (s, 18-H_3), 2.455 (d, J = 15.5 Hz, 17α-H), 2.655 (m, 6-H_2), 2.911 (d, J = 15.5 Hz, 17β-H), 3.289 (dd, J = 6.5, 15.6 Hz, 3β-H), 3.642 (m, 5-H_2), 3.782 (s, $-CO_2CH_3$), 4.814 (dt, J = 2.1, 16.1 Hz, 3α-H), 5.635 (m, 14-H), 5.686 (d, J = 12.2 Hz, 15-H), 6.833 (d, J = 7.7 Hz, 12-H), 6.924 (t, J = 7.4 Hz, 10-H), 7.120 (d, J = 7.4 Hz, 9-H), 7.191 (ddd, J = 0.9, 7.3, 7.6 Hz, 11-H), 9.562 (d, J = 1.5 Hz, 19-H), 10.644 (br s, NH); MS, m/z (relative intensity, %) 366 (M$^+$, 32), 335 (2), 334 (1), 284 (6), 274 (8), 270 (13), 257 (4), 243 (4), 229 (17), 227 (9), 215 (25), 214 (100), 182 (15), 168 (11), 154 (48), 127 (11), 115 (4), 95 (14), 77 (6), 67 (7), 41 (10; Mass measurement, Obsd. 366.1575; Calcd, for $C_{21}H_{22}N_2O_4$, 366.1578.

Fractions 29–30 obtained from the above column were combined and evaporated *in vacuo*. The fraction (0.55 g) was rechromatographed on silica gel (100 g) and elution with $CHCl_3$–MeOH (95:5) yielded a crude solid which was crystallized from MeOH to afford **5**, (98.5 mg, 0.00044%) whose physical data (m.p., UV, IR, ^1H NMR and MS) were in agreement with those reported previously.

$NaBH_4$ *Reduction of trichophylline* (**2**). Trichophylline (**2**, 10 mg) was dissolved in redistilled MeOH (10 mL) and $NaBH_4$ (10 mg) added portion wise. The mixture was stirred at room temp for 4 hr and then poured onto cold H_2O, extracted with $CHCl_3$ (5 × 10 mL), washed with H_2O and dried (Na_2SO_4). The residue (9.6 mg) obtained after evaporation of the solvent *in vacuo* was subjected to preparative TLC on silica gel, eluting with MeOH. The band at R_f 0.54 was purified to afford a white solid (**3**, 5.6 mg); UV, λ_{max} (EtOH) 232 (log ϵ 3.96), 306 (3.97), 355 (4.12) nm; ^1H NMR, δ (360 MHz, $CDCl_3$) 1.162 (s, 18-H_3), 2.323 (d, J = 14.5 Hz, 17α-H), 2.796 (dd, J = 2.8, 14.5 Hz, 17β-H), 2.83 (m, 6-H_2), 3.60 (m, 5-H_2), 4.031 (d, J = 10.9 Hz, 19α-H), 4.05 (m, 3-H_2), 4.232 (dd, J = 2.8, 10.9 Hz, 19β-H), 5.799 (d, J = 12.4 Hz, 14-H), 5.902 (m, 15-H), 6.859 (d, J = 7.8 Hz, 12-H), 6.946 (t, J = 7.5 Hz, 10-H), 7.162 (d, J = 7.4 Hz, 9-H), 7.212 (dt, J = 0.6, 7.7 Hz, 11-H), 10.449 (br s, NH); MS, m/z (relative intensity, %) 336 (M$^+$, 41), 282 (2), 281 (12), 280 (26), 256 (4), 201 (12), 200 (30), 168 (9), 154 (12), 53 (13), 47 (19).

Crystallographic data and structure analysis. Trichophylline crystallized in the orthorhombic space group $P2_12_12_1$ unit cell constants a = 6.655(3), b = 14.429(6), c = 18.797(10) Å and U = 1805 Å.[3] The density measured by floatation in KI/H_2O was 1.348 g/cm^3, and the crystal showed z = 4 molecules in the unit cell. The F (000) value was 776 and μ(MO-K_α) was 1.02 cm^{-1}.

Cell dimensions were derived from least squares treatment of the setting angles of 25 reflections. For intensity measurements, reflections were surveyed in the range $\theta \leq 26°$ and 1777 satisfied the criterion $I > 2.5\sigma$ (I) and were used in subsequent calculations.

The crystal structure was elucidated with a direct-phasing program developed by Dr. C. J. Gilmore. After preliminary adjustment of the positional and thermal parameters of the C, N and O atoms, the H atoms were located in difference electron-density maps and included in the structure-factor calculations with U = 0.07. Least squares adjustment of the parameters of the C, N and O atoms converged at $R = 0.048$, $R_w = 0.066$, with weights given by $w = 1/\sigma^2$ (/F/). Calculations were performed on a SEL 32/27 computer with programs developed at the University of Glasgow.

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