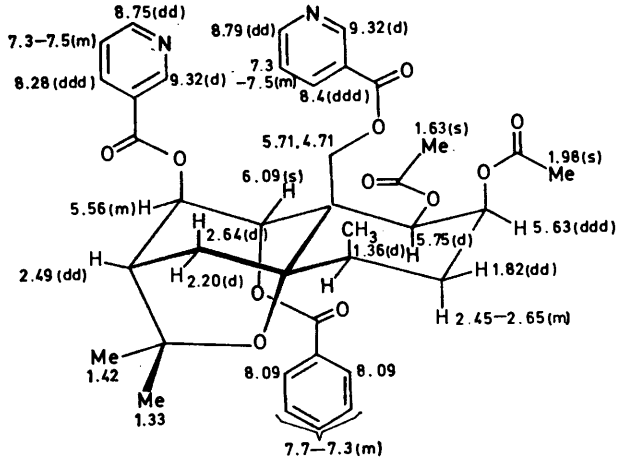
Alkaloids of *Catha edulis*. Part 2.[1] Constitution of Cathedulins E2 and E8, Polyesters of a New Sesquiterpene Pentaol

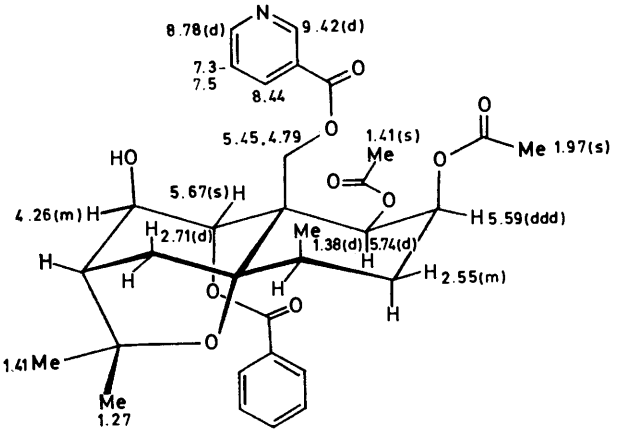
By **Robert L. Baxter, Leslie Crombie,* Derek J. Simmonds,** and **Donald A. Whiting,*** Department of Chemistry, University of Nottingham, Nottingham NG7 2RD

Cathedulin E2 yields on ethanolysis a new 1-eq, 2-ax, 8-ax, 9-ax, 15-pentahydroxylated agarofuran, as deduced from ^1H, ^{13}C, and nuclear Overhauser n.m.r. data, together with cyclic carbonate formation. Ethyl acetate, benzoate, and nicotinate in the molar ratio 2 : 1 : 2 are also produced. Graded alcoholysis (methanolic triethylamine, 5°C) gives an 8-denicotinoyl derivative identical with cathedulin E8 from *Catha edulis*, whilst at 25 °C the 8,15-bis-(denicotinyl) derivative is produced. Aqueous-methanolic sodium hydrogencarbonate further strips the 1- and 2-acetate residues leaving only the 9-benzoate ester. The placing of these ester residues is deduced from a study of the spectral changes caused by graded hydrolysis, thus leading to complete structural proposals for cathedulin E2 and E8.

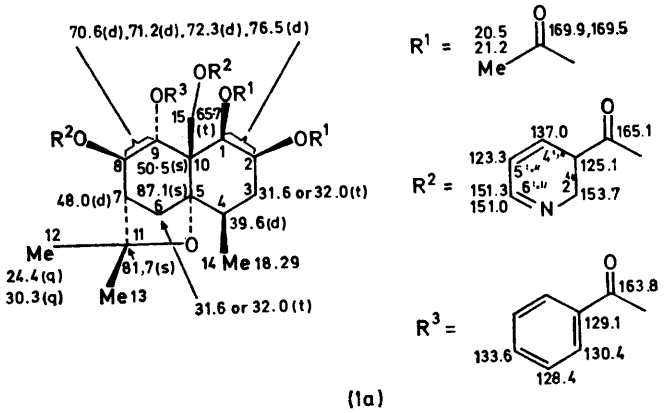
LEAVES of *Catha edulis* (Forsk) provide a drug, khat, of importance in areas such as Ethiopia, Somalia, and the Yemen. In Part 1 we have discussed the complex mixtures of alkaloids which were obtained from leaf samples collected in various localities.[1] Here, interest is focused on two related alkaloids of the group, cathedulin E2 and cathedulin E8,† which are shown to be esters (1) and (2) derived from a new pentahydroxysesquiterpene ether (3). No other derivatives of (3) have, up to the present, been identified in khat specimens.



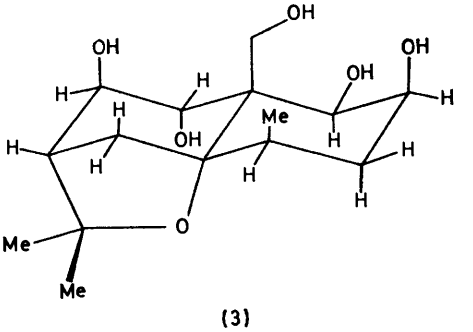
(1)



(2)



(1a)

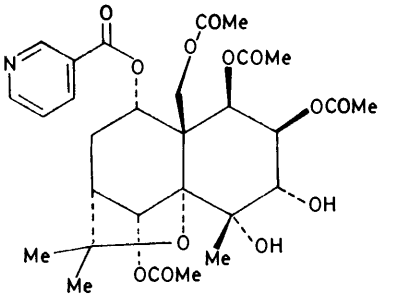


(3)

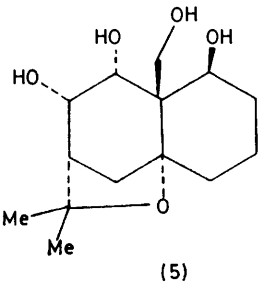
The major alkaloid of the two (cathedulin E2, 0.002 5%) was eventually obtained crystalline after extensive purification (p.l.c.). A molecular weight of 700 was indicated by mass spectrometry, using both electron impact and field desorption techniques, though even with the latter method, substantial fragmentation

† The constitutions of these alkaloids have been reported on in a preliminary communication;[2] the letter 'E' has now been added to the names employed there, in order to distinguish their Ethiopian origin.

to ions at *m/e* 685 and 640 was noted. Accurate mass measurement permitted determination of the molecular formula as $C_{38}H_{40}N_2O_{11}$, agreeing with the carbon and hydrogen atom counts as deduced from the respective n.m.r. spectra. The mass-spectral data also suggested the presence of benzoate (*m/e* 105.033, C_7H_5O), nicotinate (*m/e* 124.039, $C_6H_6NO_2$, and 106.030, C_6H_4NO), and acetate (*m/e* 43, $M^+ - 60$) units in the structure. In confirmation, ethyl acetate, ethyl benzoate, and ethyl nicotinate in the molar ratio 2 : 1 : 2 were formed on ethanolysis (g.l.c. analysis). The appropriate characteristic ^1H and ^{13}C n.m.r. resonances were also located [see (1) and (1a)].

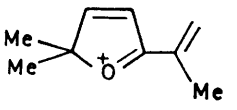


(4)



(5)

Ethanolysis provided the core pentaol of the polyester, m.p. 176—179 °C, $C_{15}H_{26}O_6$. Consideration of the ^1H and ^{13}C n.m.r. data on cathedulin E2 [displayed in full in (1) and (1a)] reveals that this pentaol core has three *C*-methyl groups, one of them being attached to –CH–, no sp^2 carbons, two quaternary carbons bound to oxygen (ether bridge), four secondary and one primary alcohol functions, and two methylene, two methine, and one quaternary carbon. These data sustain a pentahydroxy tricyclic sesquiterpene ether formulation. In recent years the Celastraceae have been shown to contain a small range of such compounds,[3] occurring as esters, *e.g.* maytoline [4] (4) and malkanguniol (5).[5] The sesquiterpene unit is, in all the cases so far reported, an hydroxylated dihydroagarofuran. In the present case, the n.m.r. data are in complete accord with the assignment of a previously unknown 1,2,8,9,15-pentahydroxydihydroagarofuran structure (3) to the pentaol core of cathedulin E2. We have not found it possible to rationalise the accumulated spectral data, which include chemical shifts, couplings and nuclear Overhauser and long-range shielding effects, by any alternative structure. Electron-impact induced fragmentation of (3) produces as base peak the ion $C_9H_{13}O$ (*m/e* 137.097), and this is also a major ion in the fragmentation of (5) and its relatives:

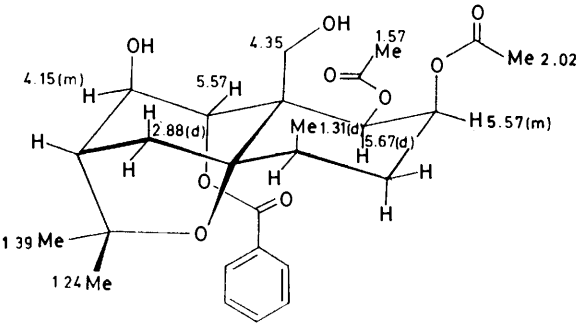


(6)

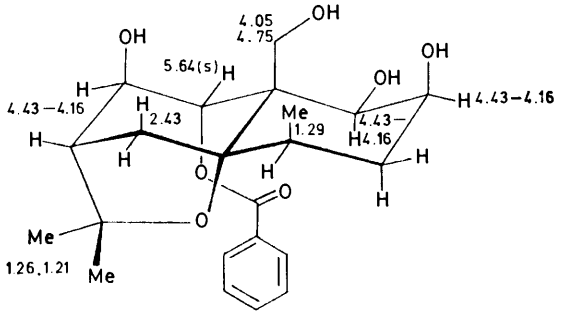
structure (6) has been advanced for it.[5] The ^{13}C n.m.r. assignments, shown in (1a), compare well with reported values in similar systems.[6]

Allocation of the esterifying acids to their respective sites was achieved by selective deacylation techniques. Treatment of (1) with methanolic triethylamine at 5 °C gave the 8-denicotinyl derivative (2), $C_{32}H_{37}NO_{10}$, in which the 8-H signal has shifted from δ 5.56 in (1) to 4.26; other data are shown in (2). This compound was found to have spectra identical to those of cathedulin E8, and the identity of the two samples was confirmed by careful t.l.c. comparison. Cathedulin E8 thus has structure (2). However, its status must remain equivocal, since the 8-nicotinyl group of E-2 is obviously labile, and E-8 could be an artefact of chromatography or the extraction procedure.

Incubation of cathedulin E2 with methanolic triethylamine at 25 °C gave a little (2) and, as the major product, the bis(denicotinyl) derivative (7), $C_{26}H_{34}O_9$, whose ^1H n.m.r. spectrum showed a 15-H_2 signal at δ 4.35 clearly pertaining to a free hydroxymethylene group. Hydroly-



(7)

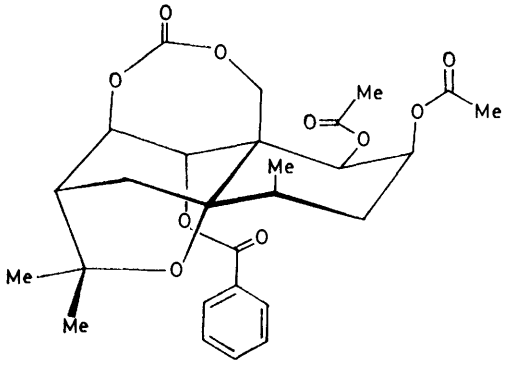


(8)

sis of E-2 with sodium hydrogencarbonate in aqueous methanol produced the tetrahydroxymonobenzoate (8), $C_{22}H_{30}O_7$. Only one proton with a chemical shift appropriate to C*H*–OCOR was observed, (δ 5.64), with the multiplicity required for the 9-H. The benzoate ester thus engages the 9-OH, and the two acetate groups must be placed at 1-OH and 2-OH.

In the hydrolytic sequence (1)→(2)→(7)→(8) some interesting changes in chemical shift were observed apart from the most obvious C*H*–OCOR ⟶ C*H*–OH effects. Thus 6-H_{ax} is increasingly deshielded on passing from (1)→(2)→(7), with the freeing of the 8- and 15-hydroxys which are spatially close. However, the deshielding is partly removed in the (7)→(8) change, suggesting a change in conformation of the 15-OH (*e.g.* to hydrogen-bond with the 2-OH rather than the 8-OH). Such a change would accord with the marked alteration in equivalence of the 15-methylene protons found in the change (7)→(8). One 3-H_2 proton is more deshielded than the other and can be observed in some spectra [δ 1.82 in (1), 2.55 in (2)]; since its signal is responsive to α-face de-esterification it is probably 3α-H, *i.e.* equatorial.

The stereochemistry assigned to (1) follows mainly from spectroscopic evidence, particularly the coupling constants; assignment of the couplings of the core ring-protons was checked by double-irradiation experiments. The 2-H is indicated as equatorial from its small couplings to 1-H and 3-H_2, and a *cis*-1H,2H relationship is suggested by *J* 1, 2, and 3.5 Hz since a narrow range of 3.2—3.5 Hz is observed for the same stereochemistry in related structures.[6] In nuclear Overhauser experiments, close spatial relationships between 14-H_3 and 15-H_2 (16% nOe) and between 14-H_3 and 6-H_{ax} * (18% nOe) were indicated, and these are only possible when C-14, C-15, and 6-H_{ax} are all axial in a *trans*-fused decalin. The compound (7) (8,15-diol) was treated with phosgene, and formed a cyclic carbonate derivative



(9)

(9), as shown by mass spectroscopy. The 8-OH must therefore be axial. With the near-zero 8-H_{eq}, 9-H coupling, the latter must also be equatorial, thus completing the stereochemical assignments. Positive shielding of the 1-acetate methyl in (1) (δ 1.63), (2) (1.41), and (7) (1.57) may arise from the neighbouring 9-axial benzoate and is parallel to an effect observed in maytoline.[4]

EXPERIMENTAL

For general procedures, see Part 1.[1]

Ethanolysis of Cathedulin E2.—The alkaloid (14 mg) was dissolved in ethanolic sodium ethoxide (0.1M, 1 cm³) and the solution set aside at room temperature for 18 h, when it was acidified with a little acetic acid, and evaporated to dryness under a stream of dry nitrogen. The residue was washed with ether (2 × 1 cm³) and separated by p.l.c. on silica using ethyl acetate–methanol (5 : 1) as eluant. The major component was isolated, and crystallised from chloroform–light petroleum to yield the *pentahydroxy-terpenoid ether* (3), 2.4 mg, 40%), m.p. 180—183 °C (decomp.) (Found: M^+, 302.175; $M^+ - 18$, 284.165. $C_{15}H_{26}O_6$ requires 302.174; $C_{15}H_{24}O_5$ requires 284.162); $\lambda_{max.}$ (MeOH) 210 nm (end absorption, ε *ca.* 3 540); $\nu_{max.}$ ($CHCl_3$) 3 400(br), 3 000, 1 640w, 1 620w cm⁻¹; δ($CDCl_3$) 4.6—3.7 (6 H, 6 × C*H*-O), 1.50 (3 H, s, 12-H_3), 1.25 (3 H, d, *J* 8.0 Hz, 14-H_3), and 1.21 (3 H, s, 13-H_3); *m/e* 302 (M^+, 2), 284 (20), 266 (15), 167 (20), 137.096 9 (100; $C_9H_{13}O$ requires 137.096 6), and 124 (65).

8-*Denicotinylcathedulin E2.*—Triethylamine (50 mm³) was added to a solution (at 5 °C) of cathedulin E2 (16 mg) in methanol (450 mm³). After 16 h at 5 °C the solution was evaporated to dryness *in vacuo*. P.l.c. of the residue, using ethyl acetate as eluant, separated two major components, unchanged cathedulin E2 (2.7 mg) and 8-*denicotinylcathedulin* (2) (7.5 mg, 65%), an amorphous colourless solid (Found: M^+, 595.243. $C_{22}H_{37}NO_{10}$ requires 595.242); $\lambda_{max.}$ (MeOH) 227 (ε 14 100), 256infl. (2 700), 264 (3 000), 270infl. (2 700), and 283infl. nm (900); $\nu_{max.}$ ($CHCl_3$) 3 400(br), 1 730(br), and 1 593 cm⁻¹; δ($CDCl_3$), 9.42 (1 H, m, 2′-H), 8.78 (1 H, br d, *J* 4.0 Hz, 6′-H), 8.44 (1 H, br, d, *J* 8.0 Hz, 4′-H), 8.06 (2 H, m, benzoate *o*-H), 7.60—7.30 (4 H, m, benzoate *m*, *p*-H and 5′-H), 5.74 (1 H, d, *J* 3.4 Hz, 1-H), 5.67 (1 H, s, 9-H), 5.59 (1 H, ddd, *J* 3.4, 3.0, and 3.0 Hz, 2-H), 5.45 (1 H, d, *J* 12.0 Hz, 15-H_a), 4.79 (1 H, d, *J* 12.0 Hz, 15-H_b), 4.26 (1 H, m, 8-H), 2.71 (1 H, d, *J* 13.0 Hz, 6-H_{ax}), 2.55 (1 H, m, 3-H_{eq}), 2.30—1.70 (4 H, m, 3-, 4-, 6-, and 7-H), 1.97 (3 H, s, COMe), 1.41 (6 H, s, COMe and 12-H_3), 1.38 (3 H, d, *J* 5.1 Hz, 14-H_3), and 1.27 (3 H, s, 13-H_3): *m/e* 595 (M^+, 2), 580 (0.5), 535 (1), 473 (2), 413 (20), 398 (2), 354 (2), 325 (45), 221 (20), 179 (10), 137 (25), 124 (50), 123 (10), 122 (5), 106 (45), and 105 (100); M^* 481 (595→535), 384 (413→398), 378 (595→473), 360 (473→413), 302 (413→354), 296 (535→398), and 264.5 (473→354).

8,15-*Bis*(*denicotinyl*)*cathedulin E2.*—Triethylamine (100 mm³) was added to a solution (at 5 °C) of cathedulin E2 (12 mg) in methanol (800 mm³) and the mixture was set aside at 25 °C for 15 h, when it was evaporated to dryness. P.l.c. of the residue as above gave 8-denicotinylcathedulin E2 (2.2 mg, 20%) and 8,15-*bis*(*denicotinyl*)*cathedulin E2* (7) (3.0 mg, 35%) as a colourless glass (Found: M^+, 490; $M^+ - 18$, 472.212. $C_{26}H_{32}O_8$ requires 490, $C_{26}H_{30}O_7$ requires 472.210); $\lambda_{max.}$ (MeOH) 233 nm (ε 13 700); $\nu_{max.}$ ($CHCl_3$) 3 400(br), 1 740, 1 710, 1 600, and 1 585 cm⁻¹; δ($CDCl_3$) 8.04 (2 H, m, benzoate *o*-H), 7.60—7.36 (3 H, m, benzoate *m*, *p*-H), 5.67 (1 H, d, *J* 3.4 Hz, 1-H), 5.57 (1 H, s, 9-H), 5.57 (1 H, obscured, 2-H), 4.35 (2 H, br s, 15-H_2), 4.15 (1 H, m, 8-H), 2.8 (1 H, d, *J* 12.0 Hz, 6-H_{ax}), 2.02

* In ref. 2, the ^{1}H n.m.r. assignments of 6-H_{ax} and 6-H_{eq} were unintentionally inverted.

(3 H, s, COMe), 1.57 (3 H, s, COMe), 1.39 (3 H, s, 12-H_3), 1.31 (3 H, d, *J* 6 Hz, 14-H_3), and 1.24 (3 H, s, 13-H_3); *m/e* 490 (0.5), 472 (10), 308 (15), 221 (10), 137 (35), 122 (40), and 105 (100).

Sodium Hydrogencarbonate Hydrolysis of Cathedulin E2.—A mixture of cathedulin E2 (15.4 mg) in methanol (1.2 cm^3) and aqueous sodium hydrogencarbonate (0.2M, 0.6 cm^3) was set aside at room temperature for 4 d. The solution was concentrated *in vacuo* and extracted with chloroform (2 × 2 cm^3). The washed, dried, organic extract was evaporated and the residue separated using repeated p.l.c. first with ethyl acetate eluant, and then with ether. In this way the minor 8,15-bis(denicotinyl) product was isolated (0.8 mg, 7.5%). The major component proved to be the *monobenzoate* (8), (4.4 mg, 50%) as a colourless amorphous solid; $\lambda_{max.}$ (MeOH) 230 nm (ε 11 300); $\nu_{max.}$ ($CHCl_3$) 3 450(br) 1 700, 1 602, and 1 590 cm^{-1}; δ($CDCl_3$) 7.95 (2 H, m, benzoate *o*-H), 7.50—7.25 (3 H, m, benzoate *m*, *p*-H), 5.64 (1 H, s, 9-H), 4.75 (1 H, d, *J* 11.0 Hz, 15-H_a), 4.43 and 4.16 (3 H, 2 m, 1-, 2-, and 8-H), 4.05 (1 H, d, *J* 11.0 Hz, 15-H_b), 2.43 (1 H, d, *J* 12.5 Hz, 6-H_{ax}), 1.29 (3 H, d, *J* 6.5 Hz, 14-H_3), 1.26 and 1.21 (both 3 H, s, 12-H_3 and 13-H_3); *m/e* 406, 388, 137, and 105.

Cyclic Carbonate of 8,15-Bis(denicotinyl)cathedulin E2.—The diol (7) (2.0 mg) in dry pyridine (150 mm^3) and methylene chloride (50 mm^3) was treated with a solution (12% w/v, 50 mm^3) of phosgene in toluene, and the mixture set aside at room temperature for 3 d. The mixture was poured into ice–water and extracted with chloroform (2 × 1 cm^3). The extracts were washed, dried, and evaporated. The residue was subjected to successive p.l.c. purifications using ethyl acetate–benzene (1:1) and then ether as eluants, to afford the *carbonate* (9) (500 μg) as an amorphous solid, giving a single spot on t.l.c.; *m/e* 516 (M^+, 2), 488 (*M* − 28, 2), 456 (*M* − 60, 15), 395 (*M* − 121, 40), 221 (15), 137 (20), 124 (30), and 105 (100).

Quantitative Transesterification of Cathedulin E2.—Cathedulin E2 (14 mg) was added to 0.1M-sodium ethoxide in ethanol (1 cm^3) held at liquid-nitrogen temperatures, and the reaction vessel was sealed. The mixture was set aside at ambient temperature for 18 h, and then analysed by g.l.c. immediately upon opening the vessel. Ethyl acetate was detected on a Carbowax 20M Column at 60 °C, and ethyl benzoate and ethyl nicotinate were detected at 160 °C on a PEGA column. Standard solutions of esters provided calibrations for the quantitative assay.

[8/1496 *Received*, 14*th August*, 1978]

REFERENCES

[1] Part 1, L. Crombie, D. J. Simmonds, D. A. Whiting, O. J. Braenden, and K. Szendrei, preceding paper.
[2] R. L. Baxter, L. Crombie, D. J. Simmonds, and D. A. Whiting, *J.C.S. Chem. Comm.*, 1976, 465.
[3] R. M. Smith, in 'The Alkaloids,' ed. R. H. F. Manske, Academic Press, London, ch. 4, p. 215.
[4] S. M. Kupchan, R. M. Smith, and R. F. Bryan, *J. Amer. Chem. Soc.*, 1970, **92**, 6667.
[5] H. J. den Hertog, J. Th. Hackmann, D. D. Nanavati, and S. Dev, *Tetrahedron Letters*, 1973, 845; H. J. den Hertog, C. Kruk, D. D. Nanavati, and S. Dev, *ibid.*, 1974, 2219.
[6] Y. Shizuri, H. Wada, K. Sugiura, K. Yamada, and Y. Hirata, *Tetrahedron*, 1973, **29**, 1773.