Alkaloids of *Catha edulis*. Part 4.† Structures of Cathedulins E3, E4, E5, E6, and K12. Novel Sesquiterpene Alkaloids with Mono- and Bis-macrolide Bridges

By **Robert L. Baxter, W. Mary L. Crombie, Leslie Crombie,* Derek J. Simmonds, and Donald A. Whiting,*** Department of Chemistry, The University, Nottingham NG7 2RD
Kalman Szendrei,* United Nations Narcotics Laboratory, Geneva, Switzerland

Alcoholysis and reduction shows that cathedulin E3, $C_{54}H_{60}N_2O_{23}$, is made up of a euonyminol core (3) (octa-acetate), together with four acetates, a hydroxyisobutyrate, and evoninate (1) and cathate residues. The latter, a novel residue, is shown to be (4). By study of ^1H and ^{13}C n.m.r. data for the intact alkaloid and its hydrogenolysis and partial methanolysis products, E3 is formulated as (12). Cathedulin E4, converted into E3 by acetylation, is (13). Corresponding to both alkaloids is a minor series in which one acetate is replaced by a benzoate residue.

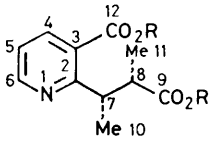
Cathedulin E6, $C_{57}H_{62}N_2O_{22}$, formulated as (17), is also based on a euonyminol core but gives, on methanolysis, dimethyl evoninate, methyl benzoate, methyl nicotinate, and trimethyl gallate; a 2-acetoxyisobutyrate and a further acetate residue are also present. Alkaloid E6 bears to E5 (16) the same relationship as does E4 to E3. The minor alkaloid cathedulin K12, $C_{54}H_{62}N_2O_{23}$, has the structure (18), similar to E3 but with the cathate span reductively cleaved: this suggests a biosynthetic connection.

THE background to the problem of the nature of the constituents of *Catha edulis* (khat) was laid out in Part 1,[1] along with details of the substances isolated in the present work. In Parts 2[2] and 3[3] evidence was presented for the constitutions of two sub-sets of natural alkaloids, and in the third paper on this phase of the investigation we now discuss the structures of the remaining, and most complex, bases. These are cathedulins E3, E4, E5, and E6 from Ethiopian khat, and cathedulin K12 from a Kenyan sample. The two alkaloids mentioned first are closely interrelated and will form the first subjects for discussion.

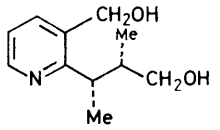
RESULTS AND DISCUSSION

Cathedulins E3 and E4 have molecular formulae $C_{54}H_{60}N_2O_{23}$ and $C_{52}H_{58}N_2O_{22}$ respectively, from electron-impact mass spectrometry. These data accord with the numbers of carbons and hydrogens counted in ^{13}C and ^1H n.m.r. spectra. Early work with these compounds was made confusing by the appearance of low intensity (*ca.* 5%) ions at $M + 62$, which persisted in samples extensively purified by p.l.c. and recrystallised. The weight of other evidence (mass spectrometry of derivatives, n.m.r. spectra, chemical degradations) points, however, to unseparated minor alkaloids as the source of the $M + 62$ ions; such compounds apparently have one acetate residue replaced by a benzoate, thus accounting for the mass difference. Acetylation of E4 forms E3 exclusively, while partial methanolysis of E3 gives a reasonable yield of E4. Alcoholysis of the alkaloids was extremely informative. Thus methanolysis of cathedulin E4 gave dimethyl evoninate (1; R = Me), known as a degradation product of evonine [4] and recognised by comparison with a specimen prepared from evonine. Ethanolysis of alkaloid E3 gave the diethyl ester (1; R = Et), and lithium aluminium hydride treatment of E4 led to evoninyl alcohol (2). Each of these scission reactions also gave euonyminol (3; R = H), not easily characterised as the highly water-soluble and polar nonaol, but readily recognised as octa-acetate (3; R = Ac) by comparison of its ^1H n.m.r. spectrum with the published trace.[4] The molecular ion (702) was obtained by field-desorption mass spectrometry; a strong $M - 17$ fragment was satisfactorily mass measured.

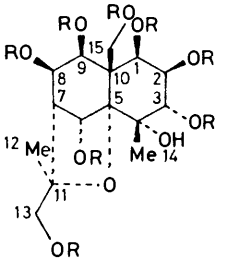
A third product of alcoholysis was the diester (4). The dimethyl compound (4; R = Me) from E4 methanolysis, a crystalline solid, $C_{18}H_{19}NO_7$, showed ^1H n.m.r. resonances arising from two ester methyls, two equivalent



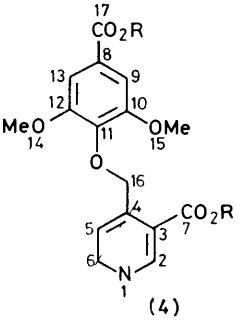
(1)



(2)



(3)



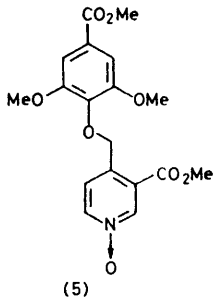
(4)

The numbering used in ciphers 1, 3, and 4 is transferred to the intact alkaloids, with the addition of primes: derivatives of (1) single primes, of (4) double primes.

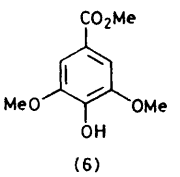
aryl methoxy-methyls, two equivalent aryl protons *ortho* to a carbonyl function (δ 7.31), a 3,4-disubstituted heteroaryl ring [δ 9.16 (s, 2-H); 8.77 (d, 6-H); and 8.08 (d, 5-H)], and an *O*-methylene whose protons were coupled to the signal at δ 8.08 (pyridine 5-H) (double resonance). These data define the structure. In further support (4; R = Me), now termed dimethyl cathate, gave an *N*-oxide (5), and on reduction with Raney nickel gave methyl syringate (6). Lithium aluminium hydride

† Part 3 is ref. 3.

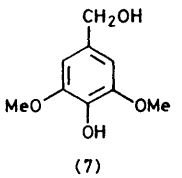
reduction of E4 provided, among the products, syringyl alcohol (7).



(5)

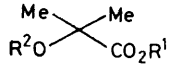


(6)



(7)

Cathedulin E3 thus contains a euonyminol core esterified by evoninate and cathate moieties. The ^{1}H n.m.r. spectrum (see below) reveals acetate methyl absorptions, and quantitative ethanolysis (g.c. analysis) showed four acetate units to be present. Further, proton signals from four quaternary C-methyl groups were present, two more than in the sesquiterpene nucleus; the presence of a 2-alkoxyisobutyrate unit as found in cathedulins K1, K2, K6, and K15 [3] was suggested, and confirmed by the detection of ethyl 2-hydroxyisobutyrate (8; R^1 = Et, R^2 = H) (g.c.) and



(8)

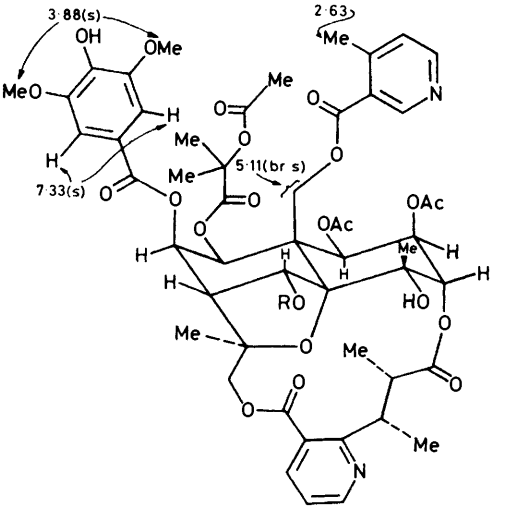
2-hydroxyisobutyric acid (g.c., t.l.c.) in the ethanolysis products of E3. This ester was detected with some difficulty, as described before.[3]

The alkaloid cathedulin E3 thus has the nonaol (3; R = H) as core, esterified by two diacids (cathic and evoninic), 4 mol equiv. of acetic acid, and 1 mol equiv. of 2-alkoxyisobutyric acid: this description accounts in full for the formula $C_{54}H_{60}N_2O_{23}$. All 60 protons can be assigned in the ^{1}H and all 54 carbons in the ^{13}C n.m.r. spectra (complete details of spectra are given in Part 1). The structural problem is thus reduced to the correct allocation of the esterifying acids to the individual hydroxy-functions, and this question is now examined.

Alkaloids E3 and E4 both contain a free tertiary hydroxy at C-4 (δ 4.57 and 5.84 respectively) in common with all known Celastraceae alkaloids. This function is hindered and not readily acylated in any compound of this type, as shown for example by the isolation of the octa-acetate only (3; R = Ac) after prolonged acetylation of the nonaol (3; R = H). In addition, E4 has a free 6-OH [δ 6.06 (d, O*H*) and 4.66 (d, 6-H), the mutual coupling being shown by double irradiation]. This group is acylated in E3 in which 6-H resonates at δ 6.0: the conversion of E4 to E3 by acetic anhydride shows the 6-OH in E3 to carry one of the acetate functions. In both compounds, a second acetate must, since 4-OH is free, be located on the 2-alkoxyisobutyrate unit which is thus present as a 2-acetoxyisobutyrate unit in these alkaloids.

The evoninate dilactone bridge is placed between 13-OH and 3-OH to accord with its position in other *Euonymus* alkaloids; this biogenetic placing is experimentally supported by the close correspondence of the n.m.r. data of the alkaloids, particularly such features as $J_{7',8'}$ *ca.* 0, indicating the H–C(7′)–C(8′)–H torsion angle to be near 90° when the section is contained in the 13—3 bridge (*cf.* *J* 9 Hz in dimethyl evoninate), the very marked separation (>2 p.p.m.) of the 13-methylene protons notable in all the compounds with this bridge intact (see below), and the shielding of 8′-H.

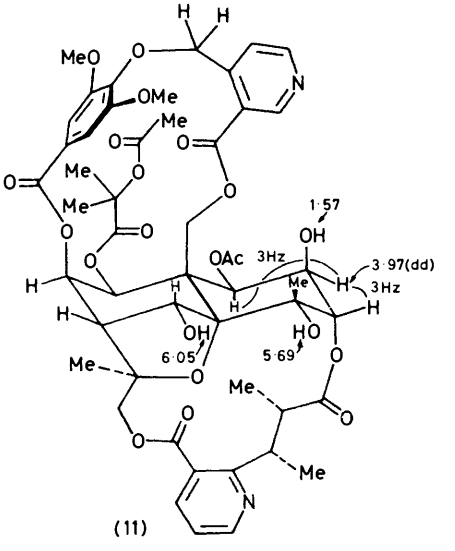
The cathate unit forms a second dilactone bridge in both E3 and E4. This bridge can be opened by hydrogenolysis over palladium, or by Raney nickel treatment, to give dihydro-E3 (9) and dihydro-E4 (10). Some



(9); R = Ac

(10); R = H

(^{1}H n.m.r. for 9)



(11)

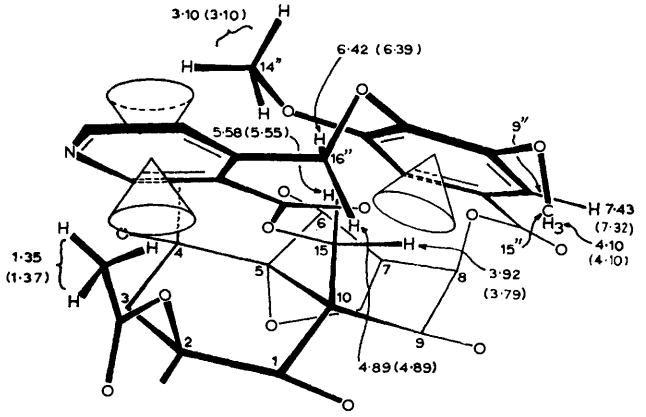
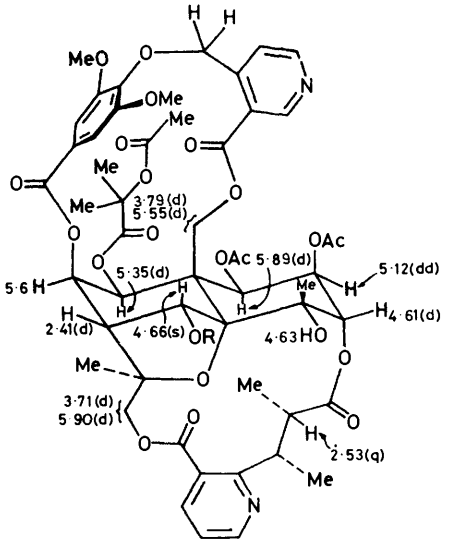


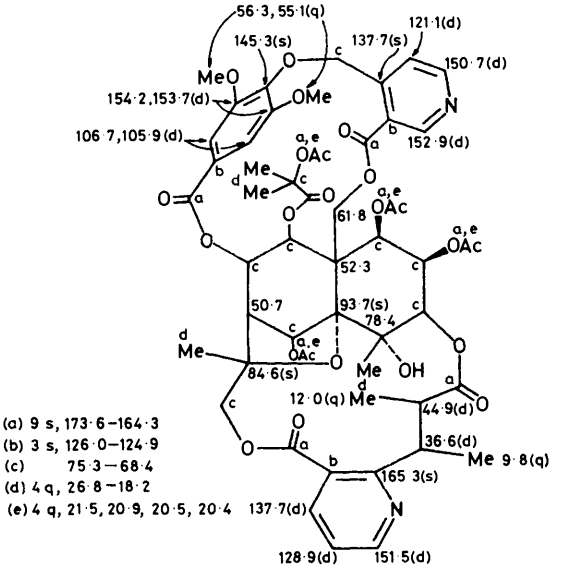
FIGURE 1 Conformation of the cathate bridge. ^{1}H n.m.r. data for E3 (E4 in parentheses)

relevant spectroscopic details are shown for (9). The new phenolic group was characterised by the reversible bathochromic shift in the u.v. on addition of alkali, and the aryl C-methyl resonated at δ 2.63. Notably, the AB signal of the 15-H_2 in E3 (δ 3.79, 5.55) collapsed to a broad singlet, suggesting clearly that one end of the cathate bridge is attached to the 15-O. For stereochemical reasons the other terminus is likely to be at the axial 8-O or the axial 2-O. The 2-O can, however, be shown to engage an acetate, since treatment of E4 with methanolic hydrazine leads smoothly to the 2-deacetylated product (11), characterised by the upfield shift of 2-H from δ 5.12 to 3.97, and the appearance of a third OH resonance. The cathate bridge thus spans 8-O_{ax}—15-O. Evidence for the orientation of the bridge comes from the

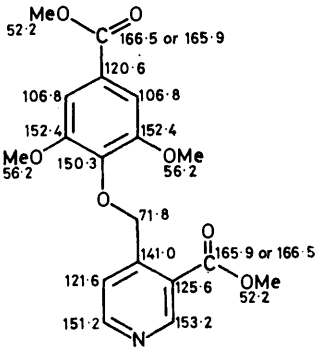


(12) R = Ac
(13) R = H

^{1}H n.m.r. spectra. Intact alkaloids E3 and E4 show, in certain resonances of the cathate bridge and its environs, striking instances of long-range shielding and deshielding as follows. (*i*) Of the two methoxy-resonances in the cathate, one is strongly positively shielded (14″-H_3, δ 3.10; *cf.* 15″-H_3, 4.10);* (*ii*) of the two ether-methylene protons of the cathate bridge one is deshielded(16″-H_A, δ 6.42; *cf.* 16″-H_B 4.89); (*iii*) one aromatic proton is relatively deshielded (9″-H, δ 7.43; 13″-H, 6.96); (*iv*) one of the methylene protons at the C-15 cathate junction is positively shielded (15-H_B, δ 3.92; *cf.* 15-H_A, 5.58); and (*v*) the 2-acetyl methyl resonates at high field, δ 1.35. We have found it possible to explain all these long-range magnetic effects only on the basis of the orientation of the cathate bridge shown in (11)—(15) in a conformation close to that displayed in Figure 1. This conformation has no compressed van der Waals contacts. In it, the cone of positive shielding of the syringate-related aromatic ring encompasses 15-H_B, while the ester carbonyl attached to the same ring, and in plane with it, deshields 9″-H. The proton 16″-H_A lies in-plane with the



^{13}C n.m.r. of cathedulin E.3.



^{13}C n.m.r. of dimethyl cathate

FIGURE 2

* N.m.r. data for E3.

pyridine-based ring, the positive shielding zone of which shields both 14″-H_3 and the 2-acetyl methyl Recognition of the last-named signal stems from the cleavage, as above, of the 2-acetyl by methanolic hydrazine to yield (11).

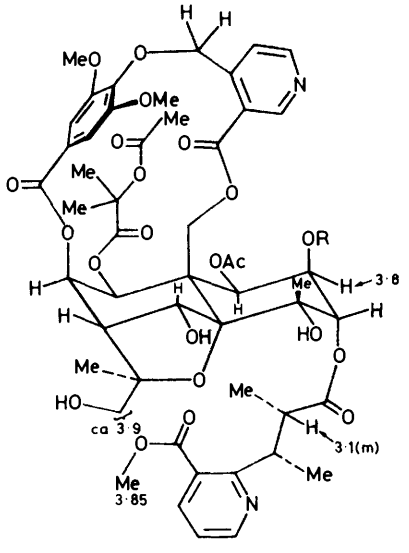
The remaining groups requiring allocation are the 2-acetoxyisobutyrate and the last acetate, while the available sites are the equatorial hydroxys at C-1 and C-9. A careful study of the deacylation sequence for cathedulin K2 showed that in that alkaloid, 2-acetoxyisobutyrate was the ester sited at C-9. It is likely, on biogenetic grounds (all alkaloids occurring in the same species), that the 2-acetoxyisobutyrate is also at C-9 in cathedulin E3 and E4 and no n.m.r. data contradict this view. The full structures of alkaloids E3 and E4 are then given in (12) and (13). Some ^{1}H n.m.r. data, for E4, are shown, and the coupling constants, supporting stereochemistry, are given in the Table. ^{13}C N.m.r.

Coupling constants (Hz) for cathedulin E4

$J_{1,2}$	3.4	$J_{13,13}$	12.0	$J_{7',10'}$	7.1
$J_{2,3}$	3.0	$J_{15,15}$	12.0	$J_{7',8'}$	0
$J_{6,7}$	*ca.* 0	$J_{4',5'}$	7.8	$J_{5'',6''}$	4.9
$J_{6,6\text{-OH}}$	3.2	$J_{5',6'}$	4.9	$J_{2'',6''}$	0
$J_{7,8}$	3.7	$J_{4',6'}$	1.8	$J_{9'',13''}$	1.8
$J_{8,9}$	5.8	$J_{8',11'}$	7.1	$J_{16'',16''}$	11.0

data, supplementing those given in Part 3, are displayed in Figure 2.

Investigation of partial methanolysis of E3 and E4 was limited. It has been shown that E3 loses the 6-acetyl most readily, and that the resulting E4 yields (11) with

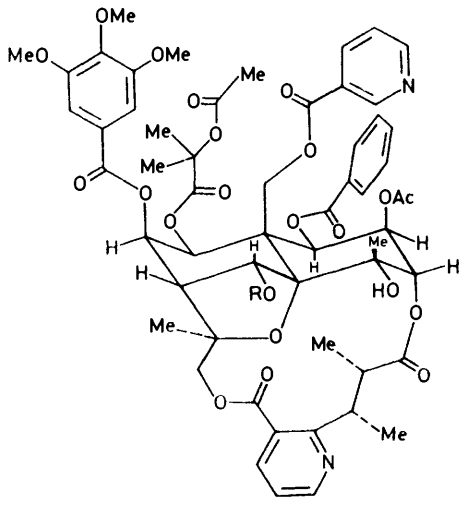


(14) R = Ac
(15) R = H

^{1}H n.m.r. data for (15)

methanolic hydrazine. With the more strongly basic medium, methanolic sodium methoxide, E4 gave a mixture of (14) and (15); *i.e.* with cleavage of C-12′–O and/or 2-acetyl.

Cathedulins E5 and E6 are related to each other as is E3 to E4. Molecular formulae $C_{59}H_{64}N_2O_{23}$ (*M* 1168) and $C_{57}H_{62}N_2O_{22}$ (*M* 1126) were indicated by electron-impact mass spectrometry, with no ions $>M^+$. Alkaloid E5 was present in very small quantity, and was

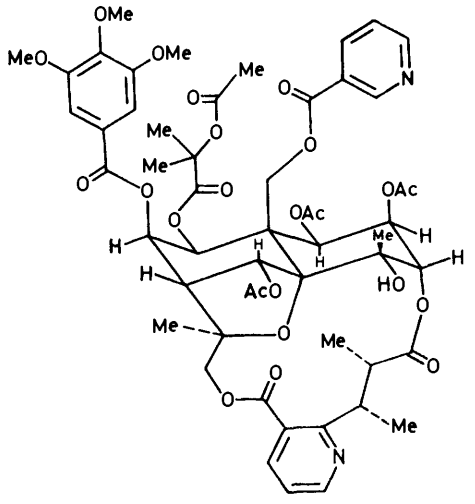


(16) R = H
(17) R = Ac

separable from E6 only with great difficulty. Accurate n.m.r. data could not be measured for E5 and its constitution rests on its production by acetylation (6-OH) of E6. Cathedulin E6 was subjected to methanolysis, and euonyminol was detected as its octa-acetate; an (equimolar) mixture of dimethyl evoninate, methyl benzoate, methyl nicotinate, and methyl trimethylgallate were also formed, detected, and analysed by g.l.c. The n.m.r. spectra show the presence of (*i*) a 2-acetoxyisobutyrate unit; (*ii*) a further acetate unit at δ 1.29, *i.e.* positively shielded; and (*iii*) free 6-OH and 4-OH (6-H, δ 4.53, 6-OH, 5.7; 4-OH, 5.78). Biogenetic parallels with E3 and E4 strongly suggest that the structures of E5 and E6 should be represented as in (16) and (17) in which (*i*) the cathate bridge is replaced by trimethylgallate and nicotinate residues, oriented in the same way; and (*ii*) a benzoate unit takes the place of a 1-acetate. The high-field acetate signal suggests that it is sited at C-2, the 15-nicotinate providing the positive shielding influence in a conformation similar to that adopted when part of the cathate bridge. Relatively high acetate-methyl signals have been observed in one other circumstance in *Celastraceae* alkaloids *viz.* for a 1-equatorial acetate shielded by a 9-axial aroyl group; [2] however in E5 and E6 the 9-alkoxy-function is equatorial, and this condition does not pertain.

Finally, a minor alkaloid from Kenyan khat, cathedulin K12, has been examined, having the molecular formula $C_{54}H_{62}N_2O_{23}$. It has ^{1}H n.m.r. data very similar to those for E3, but with signals for trimethylgallate and nicotinate esters instead of the cathate span, as in E5 and E6; structure (18) is proposed. Alkaloid K12 could pos-

sibly be the biosynthetic precursor of E3 since the $Ar'-O-CH_3 + R \rightarrow Ar'-O-CH_2-R'$ conversion, *in vivo*, is recognised in the biosynthesis of *e.g.* methylene dioxy-functions,[5a] rotenone,[5b] and homoisoflavonoids.[5c]



(18)

EXPERIMENTAL

For general considerations, see Part 1: physical data for the natural alkaloids are also listed there.

Transformation of Cathedulin E4 into Cathedulin E3.—Cathedulin E4 (20 mg) in dry pyridine (1 cm^3) was treated with excess of acetic anhydride for 3 d at ambient temperature and for 1 d at 60 °C. The product was diluted with aqueous sodium hydrogencarbonate and then extracted with chloroform (3 × 5 cm^3). The extracts were washed (water), dried, and evaporated. The residue was purified by p.l.c. using CE 32/A [the organic layer of chloroform–ethyl acetate (3 : 2), equilibrated with concentrated ammonia and used the same day], to afford cathedulin E3 (17.5 mg. 84%), identical (t.l.c., n.m.r., and mass-spectral characteristics) with the natural product.

Transformation of Cathedulin E3 into Cathedulin E4.—Cathedulin E3 (3.2 mg) in methanol (0.3 cm^3) was treated at 0 °C with diethylamine (20 mm^3) for 1 h, and then at −28 °C for 21 h. The whole product was separated by p.l.c. (CE 32/A) to give cathedulin E4 (1.9 mg, 62%) identical (t.l.c. and mass-spectroscopic comparisons) with authentic natural alkaloid.

Complete Methanolysis of Cathedulin E4.—Cathedulin E4 was sealed under nitrogen with 0.435M-sodium methoxide in methanol (1.5 cm^3) in a Reactivial. The reaction mixture was set aside for 12 h at ambient temperature, when the products were partitioned between benzene (5 cm^3) and water (5 cm^3). The aqueous layer was extracted again with benzene (2 × 5 cm^3) and set aside for treatment as below. The organic extracts were washed with brine, dried, and evaporated. The colourless residue (5.6 mg) was separated by p.l.c., using chloroform; two bands were eluted. The first band gave a pale yellow liquid (1.9 mg, 57%) identified as *dimethyl evoninate* (1; R = Me) (Found: M^+, 251.117. $C_{13}H_{17}NO_4$ requires M, 251.116) by detailed spectroscopic and chromatographic comparisons with a sample prepared from evonine by the literature method;[4] the ^1H n.m.r. data were also identical with those published.

The second band gave a solid, crystallising from methanol as colourless needles (1.7 mg. 35%) of *dimethyl cathate* (4; R = Me), m.p. 162—162.5 °C (Found: C, 60.1; H, 5.3; N, 3.9%; M^+, 361.118. $C_{18}H_{19}NO_7$ requires C, 59.9; H, 5.3; N, 3.9%; M, 361.116); $\lambda_{max.}$ (EtOH) 215 (ε 42 480), 267 (15 410) and 292 nm (4 390); $\nu_{max.}$ (KBr) 2 940, 1 720, 1 595, 775, and 765 cm^{-1}; δ_H ($CDCl_3$) 9.16 (1 H, s, 2-H), 8.77 (1 H, d, J 5.1 Hz, 6-H), 8.08 (1 H, br d, J 5.1 Hz, 5-H), 7.31 (2 H, s, Ar-*H*), 5.48 (2 H, s, OCH_2), 3.92 and 3.91 (both 3 H, s, CO_2Me), and 3.87 (6 H, s, 2 × OMe); δ_C 166.5 and 165.9 (both s, *C*O_2R), 153.2 (d, C-2), 152.4 (s, C-10 and C-12), 151.2 (d, C-6), 150.3 (s, C-11), 141.0 (s, C-4), 125.6 (s, C-3), 121.6 (d, C-5), 120.6 (s, C-8), 106.8 (d, C-9 and C-13), 71.8 (t. C-16), 56.2 (q, 2 × OMe), and 52.2 (q, CO*Me*); *m/e* 361(6), 330(12), 226(6), 211(24), 197(6), 183(12), 181(26), 150(100), 120(10), 92(20), 78(5), and 77(5).

The aqueous solution from the partition, above, was stirred with Amberlite (1R-120 (H form) ion-exchange resin to neutralise it. After filtration, the solution was evaporated to dryness. The residue was warmed with dry pyridine (1.5 cm^3) and the filtered solution was treated with acetic anhydride (0.5 cm^3). The mixture was set aside at ambient temperature for 4 d, when it was partitioned between aqueous 2M-sodium carbonate (10 cm^3) and chloroform (10 cm^3). The aqueous layer was extracted with chloroform (3 × 5 cm^3) and the combined organic layers washed (dilute hydrochloric acid, brine) and dried. Evaporation gave a solid (6 mg), purified by p.l.c. [ethyl acetate–n-hexane (4 : 1), bands made visible by water spraying] to yield *euonyminol octa-acetate* (3; R = Ac) (4.7 mg, 50%), m.p. 195—196 °C from hexane (lit.,[4] m.p. 192—193 °C); *m/e* 702 (M^+, too weak to measure accurately), and 685.239 (M − 17) ($C_{31}H_{41}O_{17}$ requires 685.234). The ^1H n.m.r. spectrum of this product was indistinguishable from the published ^1H n.m.r. trace.

Ethanolysis of Cathedulin E3.—(*a*) Freshly prepared sodium ethoxide in dry ethanol (0.16M, 2 cm^3) was added to cathedulin E3 (77 mg) in dry benzene (1 cm^3) and the solution was stirred under nitrogen at ambient temperature for 16 h. The product was evaporated and the residue repeatedly extracted with warm benzene. The benzene-insoluble remainder was dissolved in water and processed as in the previous experiment to yield euonyminol octa-acetate, m.p. 193—196 °C, identified by comparison with the above sample. The benzene solutions were evaporated and the products separated by p.l.c. using benzene–ethyl acetate–ethanol (8 : 8 : 1) into two fractions. The first was re-purified by p.l.c. using chloroform to provide *diethyl evoninate* (1; R = Et) 5.4 mg) as a yellowish liquid (Found: M^+, 279.150. $C_{15}H_{21}NO_4$ requires M, 279.147); $\lambda_{max.}$ (EtOH) 218 (ε 15 700), 241.5infl. (6 580), 272 (5 620), and 290 nm (5 320); $\nu_{max.}$ ($CHCl_3$) 3 020, 1 720, 1 600, 1 580, 1 530, and 740 cm^{-1}. ^1H N.m.r. absorptions were virtually the same as those for the dimethyl ester, but with δ_H 4.35 (2 H, q, J 8 Hz), 3.85 (2 H, q, J 8 Hz), 1.38 (3 H, t, J 8 Hz), and 0.98 (3 H, t, J 8 Hz) replacing the OMe resonances. The second band gave *diethyl cathate* (4; R = Et) (5.8 mg) from methanol, m.p. 133—136 °C (Found: M^+, 389,151. $C_{20}H_{23}NO_7$ requires M, 389.147); $\lambda_{max.}$ (EtOH) 215 (35 500), 265 (11 480), and 291infl. nm (3 780); $\nu_{max.}$ ($CHCl_3$), 3 000, 1 710, 1 600, and 1 565 cm^{-1}; δ_H ($CDCl_3$), 9.1 (1 H, s, 2-H), 8.75 (1 H, d, J 5 Hz, 6-H), 8.1 (1 H, br d, J 5 Hz, 5-H), 7.2 (2 H, s, 9- and 13-H), 5.45 (2 H, s, 16-H_2), 4.34 (4 H, q, J

8 Hz, 2 × OCH_2), 3.83 (6 H, s, 2 × OMe), and 1.38 (6 H, t, *J* 8 Hz, 2 × CH_2Me).

(*b*) Samples of crystalline cathedulin E3 (1—5 mg) were treated with sodium ethoxide (0.4 cm^3, *ca.* 0.2M) in ethanol in a 0.6 cm^3 Reactivial. Dodecane was added as an internal standard. Controls were set up with ethyl acetate in the same sodium ethoxide solution. Aliquots were removed from the reactions and assayed by g.l.c. as described in Part 3. Ethyl acetate was detected (co-chromatographs with authentic ester) and quantitative assay gave, as a mean result corrected for decomposition of ethyl acetate in ethoxide (see Part 3), 4.0 acetate ester linkages per molecule of cathedulin E3.

(*c*) Samples of cathedulin E3 in ethanolic sodium ethoxide used in (*b*) above for acetate assay were also analysed for ethyl 2-hydroxyisobutyrate, as described in detail in Part 3. This ester was detected (co-chromatography with authentic ester) although quantitative estimation was impossible for reasons explained before. The cathedulin samples used in ester analyses were collected, acidified, and continuously extracted with ether for 18 h. The ether solutions were evaporated and the residue was shown to contain free 2-hydroxyisobutyric acid (*i*) by g.l.c. of its trimethylsilyl derivative, and (*ii*) by t.l.c. of the free acid, as described before for cathedulin K2.

Reductive Cleavage of Cathedulin E4 with Lithium Aluminium Hydride.—Cathedulin E4 (140 mg) in dry ether (2 cm^3) and dry tetrahydrofuran (*ca.* 0.1 cm^3) was added dropwise to a stirred suspension at 0 °C of lithium aluminium hydride (140 mg) in dry ether (2 cm^3) under nitrogen. The mixture was allowed to warm to room temperature and stirring was continued for 15 h. Aqueous ammonium chloride (*ca.* 5M, 10 cm^3) was added and the mixture was filtered. Both the residue and the aqueous filtrate layer were washed with ethyl acetate, and the combined washings and ethereal filtrate were dried and concentrated to a brown gum. T.l.c. showed the presence of two major products within a complex mixture; these were isolated by repeated p.l.c. using benzene–ethyl acetate–ethanol (8 : 8 : 1), CM1, and CM4 (CM*x*, chloroform containing *x*% methanol). The first product was identified as syringyl alcohol (7) (2 mg), m.p. 132—134 °C (from benzene) (lit.,[6] m.p. 136 °C) (Found: M^+, 184.0733. Calc. for $C_9H_{12}O_4$; *M*, 184.0736); δ_H ($CDCl_3$) 6.60 (2 H, s, Ar-H_2), 5.47 (1 H, s, OH), 4.60 (2 H, s, CH_2OH), 3.90 (6 H, s, 2 × OMe), 1.57 (1 H, s, OH).

The second product was *evoninyl alcohol* (2) (5.4 mg) (Found: M^+, 195.1258. $C_{11}H_{17}NO_2$ requires *M*, 195.1259); $\nu_{max.}$ ($CHCl_3$) 3 400, 2 950, 1 580, and 1 470 cm^{-1}; δ_H ($CDCl_3$) 8.4 (1 H, m, 6-H), 7.65 (1 H, m, 4-H), 7.12 (1 H, dd, *J* 7.9 and 4.8 Hz, 5-H), 4.89 (1 H, d, *J* 12.2 Hz, 12-H_A), 4.50 (d, *J* 12.2 Hz, 12-H_B), 3.40 (2 H, d, *J* 4.4 Hz, 9-H_2), 3.24 (1 H, m, 7-H), 2.2—1.7 (2 H, br, 2 × OH), 1.59 (1 H, m, 8-H), 1.30 (3 H, d, *J* 7.2 Hz, 10-H_3), and 1.09 (3 H, d, *J* 7.2 Hz, 11-H_3).

The aqueous layer from the reaction, and the residues from extraction (dissolved in 50% aqueous acetic acid) were combined, de-ionised with Amberlite IR-120 (H form) resin, and treated as in previous experiments to yield euonyminol octa-acetate (17.4 mg).

Reductive Cleavage of Dimethyl Cathate by Raney Nickel.—A large excess of Raney nickel (Crosfield Nicat Grade 101) was decanted several times from ethanol and added to a stirred solution of dimethyl cathate (1 mg) in ethanol (100 mg^3) and kept at 90 °C for 15 min. Two such reactions were combined, filtered, and the solvent evaporated. On t.l.c. (chloroform) with 3% ferric chloride spray, only one product was detected, with the same R_F as methyl syringate.

Hydrogenation of Cathedulin E3.—Cathedulin E3 (109.7 mg) in ethyl acetate (0.5 cm^3) was injected into a pre-equilibrated suspension of palladium (5% on charcoal, 30 mg) in isopropyl alcohol under a hydrogen atmosphere in a Brown apparatus. The mixture was vigorously stirred, at ambient temperature, until hydrogen uptake ceased (140 h), when it was filtered, and solvent was removed *in vacuo*. The residue was separated by p.l.c. using two elutions with CM3. Four bands were observed: on extraction, the first gave unchanged cathedulin E3 (57 mg). Bands 2 and 3 (4.7 and 4.5 mg) gave clear gums both containing dihydro-derivatives (*M* 1 106); ^1H n.m.r. spectra were poorly resolved but suggested that partial reduction of the pyridine rings had taken place. The remaining fraction provided the *phenol* (9) (10 mg), product of benzylic hydrogenolysis (Found: M^+, 1106.384. $C_{54}H_{62}N_2O_{23}$ requires *M*, 1106.375); $\lambda_{max.}$ (EtOH) 221 (ε 33 454), 275 (12 460), and 285infl. nm (11 539); $\lambda_{max.}$ (EtOH–NaOH) 213infl. (ε 28 605), 262infl. (6 690), and 337 nm (19 151); $\nu_{max.}$ ($CHCl_3$) 3 540, 2 950, 1 745, 1 720, 1 620, and 1 565 cm^{-1}; δ_H ($CDCl_3$) 9.23 (1 H, s, 2″-H), 8.70 (1 H, dd, *J* 2 and 5 Hz, 6′-H), 8.57 (1 H, d, *J* 5.1 Hz, 6″-H), 8.09 (1 H, dd, *J* 2 and 8 Hz, 4′-H), 7.33 (2 H, s, 9″ and 13″-H), 7.3 (1 H, d, obscured, 5″-H), 7.2 (1 H, obscured, 5′-H), 6.19 (1 H, s, 6-H), 5.93 (1 H, d, *J* 12 Hz, 13-H_A), 5.81 (1 H, d, *J* 2.9 Hz, 1-H), 5.56 (1 H, m, 8-H), 5.44 (1 H, d, *J* 5.4 Hz, 9-H), 5.30 (1 H, dd, *J* 2.9 and 2.9 Hz, 2-H), 5.11 (2 H, br s, 15-H_2), 4.73 (1 H, d, *J* 2.9 Hz, 3-H), 4.60 (1 H, m, 7′-H), 4.53 (1 H, s, 4-OH), 3.88 (6 H, s, 2 × OMe), 3.70 (1 H, d, *J* 12 Hz, 13-H_B), 2.63 (3 H, br s, 16″-H_3), *ca.* 2.6 (1 H, m, obscured, 8′-H), 2.49 (1 H, d, *J* 3.9 Hz, 7-H), 2.17, 1.98, and 1.95 (each 3 H, s, OCOMe), 1.8—1.7 (12 H, br., 1 × OCOMe and 3 × Me), 1.67 (3 H, br s, CMe), 1.40 (3 H, d, *J* 7.1 Hz, 10′-H_3), and 1.2 (3 H, d, 11′-H_3); *m/e* 1 106 (M^+, 95), 1 064(10), 1 047(8), 987(7), 956(14), 942(11), 926(10), 908(12), 859(8), 849(6), 812(7), 740(11), 649(6), 640(8), 602(6), 577(5), 529(6), 502(5), 423(12), 412(22), 396(15), 383(12), 339(8), 309(36), 293(9), 283(19), 273(14), 262(8), 255(10), 239(7), 232(12), 225(13), 206 (45), 198(32), 195(34), 189(23), 181(37), 178(23), 138(30), 124(55), 120(32), 107(56), 106(48), 105(61), and 43(100).

Treatment of cathedulin E3 (0.5 mg) in ethanol at 80 °C with excess of Raney nickel for 40 min appeared, on analysis by t.l.c., to give the same hydrogenolysis product.

Reductive Cleavage of Cathedulin E4 with Raney Nickel.—Cathedulin E4 (18 mg) and Raney nickel (Crosfield 102) (50 mg) were stirred together in ethanol (2 cm^3) for 3 h at ambient temperature. The mixture was filtered and the filtrate evaporated. The residue was separated by p.l.c. (2% methanol–chloroform) to yield some unchanged alkaloid, a band containing impure unidentified material (3 mg), and, as the major product, the *phenol* (10) (5 mg, 25%) (Found: M^+, 1 064. $C_{52}H_{60}N_2O_{22}$ requires *M*, 1 064); $\lambda_{max.}$ (MeOH) 272, 288, and 290infl. nm; $\lambda_{max.}$ (MeOH–NaOH) 265, 272, and 333 nm; δ_H ($CDCl_3$–D_2O) 9.21 (1 H, s, 2″-H), 8.70 (1 H, dd, 6′-H), 8.57 (1 H, d, 6″-H), 7.39 (2 H, s, 9″- and 13″-H), *ca.* 7.3 (5′-H, 5″-H), 2.66 (3 H, s, 16″-H_3), 2.00, 1.78, and 1.77 (each 3 H, s, OCOMe), 1.71 (12 H, br s, 4 × CMe), 1.41 (3 H, d, *J* 7 Hz, 10′-H_3), and 1.19 3 H, d, *J* 7 Hz, 11′-H_3). The weakness of ^1H n.m.r. resonances (limitation of sample mass) prevented precise measure-

ments of the overlapped sesquiterpenoid core protons, although the expected general features were observed.

Partial Methanolysis of Cathedulin E4.—(*a*) Cathedulin E4 (12.5 mg) in methanol (0.8 cm^3) containing hydrazine hydrate 70 mm^3, 98—100%) was set aside at 0 °C for 15 h. The mixture was diluted with water (5 cm^3) and extracted with chloroform (3 × 5 cm^3). The extracts were washed (brine), dried, and evaporated. The residue (*ca.* 7 mg) was separated by p.l.c. (CM4) into unchanged alkaloid E4 (2.1 mg) and the major product, 2-*deacetylcathedulin E4* (11) (5.2 mg, 53%) (Found: M^+, 1 020.343. $C_{50}H_{56}N_2O_{21}$ requires M, 1 020.338); $\lambda_{max.}$ (EtOH) 215 (ε 32 400), 267 (11 700), and 295infl. nm (5 410); $\nu_{max.}$ ($CHCl_3$) 3 410, 2 920, 1 735, 1 715, 1 705, 1 595, 1 565, and 1 500 cm^{-1}; δ_H ($CDCl_3$) 9.27 (1 H, s, 2″-H), 8.7 (2 H, dd and d, 6′ and 6″-H), 8.13 (1 H, dd, *J* 2 and 8 Hz, 4′-H), 7.60 (1 H, d, *J* 5.4 Hz, 5″-H), 7.30 (1 H, d, *J* 2 Hz, 9″-H), *ca.* 7.2 (obscured, 5′-H), 7.00 (1 H, d, *J* 2 Hz, 13″-H), 6.35 (1 H, d, *J* 11 Hz, 16″-H_A), 6.05 (1 H, d, *J* 3 Hz, 6-OH), 5.92 (1 H, d, *J* 12 Hz, 13-H_A), 5.69 (1 H, s, 4-OH), 5.68—5.50 (3 H, m, 1,8,15-H_A), 5.32 (1 H, br d, *J* 6 Hz, 9-H), 4.83 (1 H, d, *J* 11 Hz, 16″-H_B), *ca.* 4.8 (1 H, m, 7′-H), 4.64 (1 H, d, *J* 3 Hz, 6-H), 4.56 (1 H, d, *J* 3 Hz, 3-H), 4.08 (3 H, s, 15″-H_3), 3.97 (1 H, t, *J* 3 Hz, 2-H), 3.69 (2 H, br d, *J* 12 Hz, 13-H_B and 15-H_B), 3.11 (3 H, s, 14″-H_3), *ca.* 2.5 (1 H, m, 8′-H), 2.42 (1 H, d, *J* 4 Hz, 7-H), 2.14 (6 H, s, 2 × OCOMe), 2.00, 1.83, 1.76, and 1.66 (each 3 H, C–CH_3), 1.57 (br s, O*H*), 1.32 (3 H, d, *J* 7 Hz, 10′-H_3), and 1.07 (3 H, d, *J* 7 Hz, 11′-H_3); *m/e* 1 020 (40, M^+), 1 002 (2), 990 (2), 870 (2), 856 (3), 831 (2), 737 (3), 402 (4), 316 (7), 262 (5), 206 (100), 204 (10), 198 (22), 181 (57), 178 (18), 162 (13), 161 (17), 160 (26), 151 (7), 150 (6), 146 (6), 138 (23), 137 (6), 132 (8), 130 (8), 117 (9), 107 (37), 106 (7), 93 (6), and 78 (5).

(*b*) Cathedulin E4 (58.1 mg) in dry methanol (0.8 cm^3) was cooled to 0 °C, methanolic sodium methoxide (1 cm^3) was added, and the mixture set aside at 0 °C for 2.5 h. Water (10 cm^3) was added and the product extracted with chloroform (6 × 10 cm^3). The aqueous part was treated as described above to detect euonyminol octa-acetate. The bulked organic layers were washed, dried, and evaporated to afford a yellow gum (59 mg), subjected to p.l.c. using CM4. Dimethyl evoninate was recognised as one product (highest R_F). The adjacent band provided the *methyl ester* (14) derivative, an amorphous solid (15 mg) (Found: M^+ 1 094.399. $C_{53}H_{62}N_2O_{23}$ requires M, 1 094.375); $\lambda_{max.}$ (EtOH) 215 (ε 39 000), 267 (12 550), and 291infl. nm (5 530); $\nu_{max.}$ ($CHCl_3$) 3 400, 3 000, 1 745, 1 720, 1 590, and 1 505 cm^{-1}; δ_H ($CDCl_3$) 9.24 (1 H, s, 2″-H), 8.78 (1 H, d, *J* 5 Hz, 6″-H), 8.47 (1 H, dd, *J* 2 and 5 Hz, 6′-H), 8.14 (1 H, dd, *J* 2 and 8 Hz, 4′-H), 7.67 (1 H, d, *J* 5 Hz, 5″-H), 7.33 (1 H, d, *J* 2 Hz, 9″-H), *ca.* 7.2 (1 H, dd, obscured, 5′-H), 6.99 (1 H, d, *J* 2 Hz, 13″-H), 6.38 (1 H, d, *J* 11 Hz, 16″-H_A), 6.04 (1 H, d, *J* 3 Hz, 1-H), 5.6—5.3 (*ca.* 3 H), 5.02 (1 H, t, *J* 3 Hz, 2-H), 4.9 (1 H, s, O*H*), 4.88 (1 H, d, *J* 11 Hz, 16″-H_B), 4.64 (1 H, d, *J* 3 Hz, 3-H), 4.57 (1 H, br s, 6-H), *ca.* 4.1 (1 H, m, 7′-H), 4.09 (3 H, s, 15″-H_3), 3.92 (3 H, s, CO_2Me), 3.92 (2 H, obscured), 3.69 (1 H, d, *J* 15 Hz, 15-H), 3.11 (3 H, s, 14″-H_3), *ca.* 3.1 (1 H, m, 8′-H), 2.95 (1 H, s), 2.36 (1 H, d, *J* 3.5 Hz, 7-H), 2.3—1.9 (2 H, m), 2.19 and 2.13 (both 3 H, s, OCOMe), 1.82, 1.78, 1.75, and 1.66 (each 3 H, s, 4 × CMe), 1.47 (3 H, d, *J* 7 Hz, 10′-H_3), 1.31 (3 H, s, 2-OCOMe), *ca.* 1.3 (3 H, d, obscured, 11′-H_3).

The next p.l.c. band gave an amorphous solid (17.1 mg), the *2-deacetylmethyl ester* (15) (Found: M^+, 1 052. $C_{51}H_{60}N_2O_{22}$ requires M, 1 052); $\nu_{max.}$ ($CHCl_3$) 3 400, 2 970, 1 730i, 1 718, 1 590, and 1 500 cm^{-1}; δ ($CDCl_3$) 9.17 (1 H, s, 2″-H), 8.56 (1 H, d, *J* 5.1 Hz, 6″-H), 8.43 (1 H, dd, *J* 2 and 5 Hz, 6′-H), 8.05 (1 H, dd, *J* 2 and 8 Hz, 4′-H), 7.49 (1 H, d, *J* 5.1 Hz, 5″-H), 7.22 (1 H, d, *J* 2 Hz, 9″-H), 7.13 (1 H, dd, *J* 5 and 8 Hz, 5′-H), 6.93 (1 H, d, *J* 2 Hz, 13″-H), 6.23 (1 H, d, *J* 11 Hz, 16″-H_A), 5.77 (1 H, d, *J* 3 H, 1-H), 5.5—5.3 (3 H), 4.72 (1 H, d, *J* 11 Hz, 16″-H_B), 4.66 (1 H, s, OH), 4.46 (1 H, d, s after D exchange, 6-H), 4.45 (1 H, d, *J* 3 Hz, 3-H), 3.99 (3 H, s, 15″-H_3), 3.85 (3 H, s, CO_2Me), 3.8—3.7 (*ca.* 3 H), 3.8 (1 H, t, 2-H), 3.47 (1 H, d, *J* 4 Hz, 7-H), 2.08 (6 H, s, 2 × OCOMe), 1.79, 1.75, 1.66, and 1.56 (each 3 H, s, 4 × CMe), 1.30 (3 H, d, *J* 7 Hz, 10′-H_3), and 1.16 (3 H, d, *J* 7 Hz, 11′-H_3).

The remaining p.l.c. bands gave mixtures of minor products which were not investigated further.

Formation of N-Oxides.—The substrates (*ca.* 0.2 mg) were dissolved in chloroform (0.5 cm^3) with *meta*-chloroperbenzoic acid (1 mg) and set aside at ambient temperature overnight. After washing with alkali, the organic solutions were evaporated, and the product (relatively polar) isolated by p.l.c. In this way, dimethyl cathate, cathedulin E4, and 2-deacetylcathedulin E4 gave the corresponding *N*-oxides, *M* 377, 1 078, and 1 036 respectively, were formed as indicated by mass spectrometry.

Acetylation of Cathedulin E6.—A sample (18.5 mg) of cathedulin E6, free of other alkaloids, was dissolved in dry pyridine (1.5 cm^3) with acetic anhydride (0.5 cm^3), and the solution stirred for 9 d at room temperature. The mixture was agitated with aqueous sodium carbonate and extracted with chloroform (4 × 5 cm^3). The organic layers were washed, dried, and evaporated. T.l.c. of the residue showed cathedulin E5 and unchanged E6 to be present. P.l.c. using E/A (5 elutions) gave a sample of E5, still containing a trace of E6, but indistinguishable and inseparable from similar mixtures of natural origin by t.l.c. in numerous solvent systems.

Methanolysis of Cathedulin E6.—(*a*) A solution of sodium methoxide in methanol (0.435M, 0.4 cm^3) was added to pure cathedulin E6 (4.8 mg) in a Reactivial. The solution was sealed under nitrogen and set aside for 12 h at laboratory temperature. T.l.c. analysis, using both ethyl acetate–n-hexane (4 : 1) and chloroform eluants, indicated the presence of methyl benzoate, methyl 3,4,5-trimethoxybenzoate, methyl nicotinate, and dimethyl evoninate; authentic esters were employed for reference. For confirmation the mixture was partitioned between ether and water. The aqueous layer was extracted with ether, and the combined ether layers were concentrated and analysed by g.l.c. with a 50-ft capillary SCOT column, OV-225 and using a temperature programme (100 °C for 25 min then rising by 49° min^{-1} to 190 °C). Four peaks at the retention times of the four esters above were observed. On admixture with a test solution of the authentic esters, no separation was observed under these g.l.c. conditions. By reference to the test mixture the four esters were seen to be obtained in (approximately) equimolar proportions.

(*b*) A second sample (33.8 mg) was treated with excess of methanolic sodium methoxide as above. The products were isolated from the aqueous layers as previously described for cathedulin E4, and the crude water-soluble material (14.2 mg) was acetylated to yield euonyminol octa-acetate (9.8 mg, 49%), m.p. 189—191 °C, with identical i.r. and n.m.r. spectra to those of authentic samples.

[8/1498 *Received*, 14*th August*, 1978]

REFERENCES

[1] Part 1, R. L. Baxter, L. Crombie, D. J. Simmonds, D. A. Whiting, O. J. Braenden, and K. Szendrei, *J.C.S. Perkin I*, 1979, 2965.

[2] Part 2, R. L. Baxter, L. Crombie, D. J. Simmonds, and D. A. Whiting, *J.C.S. Perkin I*, 1979, 2972.

[3] Part 3, L. Crombie, W. M. L. Crombie, D. A. Whiting, and K. Szendrei, preceding paper.

[4] Y. Shizuri, H. Wada, K. Sugiura, K. Yamada, and Y. Hirata, *Tetrahedron*, 1973, **29**, 1773.

[5] (*a*) D. H. R. Barton, G. W. Kirby, and J. B. Taylor, *Proc. Chem. Soc.*, 1962, 340; (*b*) L. Crombie, P. M. Dewick, and D. A. Whiting, *J.C.S. Perkin I*, 1973, 1285; (*c*) P. M. Dewick, *Phytochemistry*, 1975, **14**, 983.

[6] 'Dictionary of Organic Compounds,' Eyre and Spottiswoode, London, 1965.