New Macrolide Sesquiterpene Alkaloids of *Catha edulis*: Examples containing a Novel Dilactone Bridge

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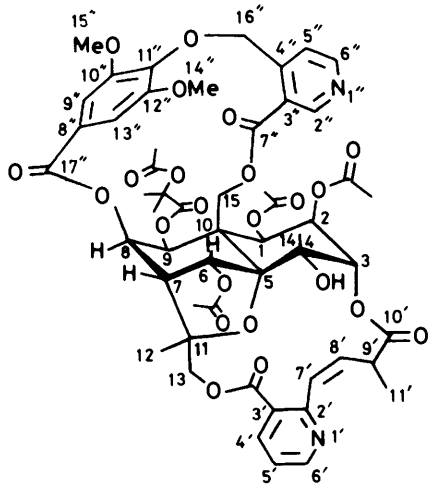
The constitutions of three new sesquiterpene alkaloids, cathedulin-K19 (**1**) ($C_{54}H_{58}N_2O_{23}$, *M* 1 102), cathedulin-K17 (**6**) ($C_{59}H_{62}N_2O_{23}$, *M* 1 166), and cathedulin-K20 (**8**) ($C_{59}H_{62}N_2O_{23}$, *M* 1 166), obtained in small quantities from stems and leaves of *Catha edulis* (khat), have been demonstrated by spectroscopic examination. Alkaloids K17 and K19 contain a novel diester bridge derived from (*Z*)-4-(3-carboxy-2-pyridyl)-2-methylbut-3-enoic acid.

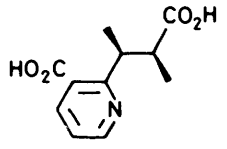
Catha edulis (Forsk) (Celastraceae) is a small tree, widely cultivated in East Africa and areas of the Middle East to provide, from fresh leaves and young shoots, a drug known as khat. The drug acts as a stimulant and suppresses appetite. The physiological effects are in part due to the cathine [(+)-norpseudoephedrine] and cathinone content, but other constituents may also be active. To assist accurate understanding of the pharmacology we have made a detailed examination of the constituents of khat, which has revealed a group of alkaloids with molecular weights *ca.* 600—*ca.* 1 200 daltons, all containing sesquiterpene polyol cores.[1-4] Neutral terpenoids and phenylalkylamines have also been investigated,[5] as well as the root-bark pigments.[1] Full background literature is given in ref. 1. We have continued work on the alkaloids of khat and in this paper report the structures of three new compounds, cathedulin-K17, -K19, and -K20, all from plant material collected in Kenya.

Cathedulin-K19 was initially isolated as an oil (87 mg) by repeated high-performance liquid chromatography (h.p.l.c.) of leaf extract (see Experimental section), though a crystalline sample, m.p. 249—251 °C, was obtained later. The molecular formula $C_{54}H_{58}N_2O_{23}$ was indicated by m.s. (FAB—fast-atom bombardment) (strong $M + 1$ ion at m/z 1 103.34) and was supported by carbon and proton counts in the n.m.r. spectra. Both ^{13}C and ^1H n.m.r. spectra showed strong resemblances to those[1] of cathedulin-E3.[4] Comparative analysis leads to the structural proposal (**1**) for cathedulin-K19.

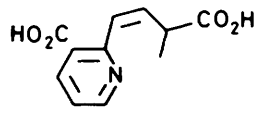


(**1**)

All the carbon and proton resonances appropriate to the euonyminol core (C-1—C-15), the cathic acid residue (C-2″—C-17″), three acetate units, and the α-acetoxyisobutyrate residue were assigned. The shifts and coupling constants (Experimental section) are very close to those of cathedulin-E3, and parallel esterification patterns in the two alkaloids appear very likely. However, cathedulin-E3 contains a 3,13-diester bridge involving evoninic acid (**2**). The n.m.r. data for the evoninate unit are replaced in cathedulin-K19 by those arising from a pyridine diacid (**3**). The pertinent ^{13}C and ^1H n.m.r data

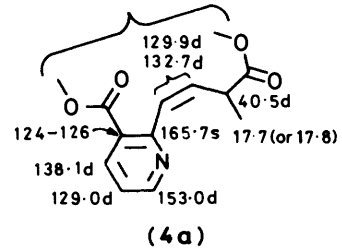


(**2**)

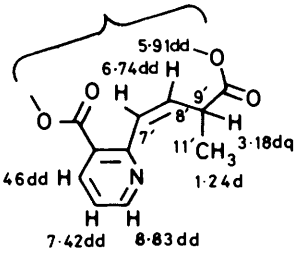


(**3**)

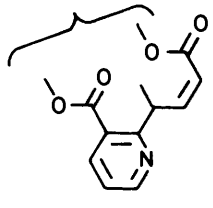
are shown in structures (**4a**) and (**4b**) respectively. The proton connectivity 7′-H–8′-H–9′-H–11′-H_3 was established by decoupling. Difference n.O.e. spectra, with irradiation at δ_H 6.74 and 5.91 in turn, showed enhancements, without decoupling, at the other signal (> 5%), thus indicating the (*Z*)-configuration



(**4a**)



(**4b**) $J_{7',8'}$ 11·0 Hz
$J_{8',9'}$ 10·7 Hz
$J_{9',11'}$ 6·8 Hz

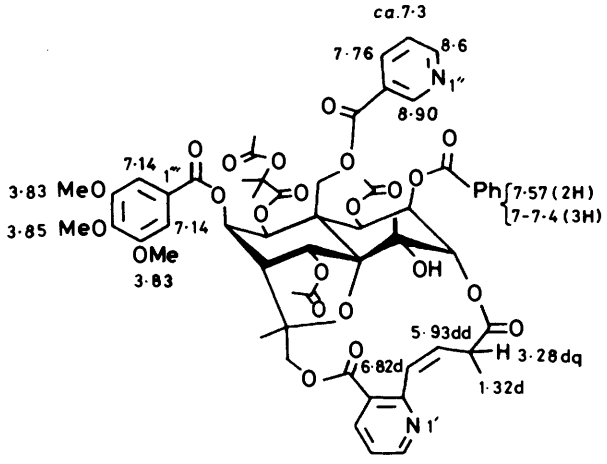


(**5**)

shown. An alternative structure (**5**) was dismissed on the grounds that the difference in chemical shift between the olefinic carbons in an α-unsaturated ester would be marked (8—12 p.p.m.) and that the olefinic proton shifts would be the reverse of the expected order.

A second alkaloid, cathedulin-K17 (**6**), was isolated as a crystalline solid (2.5 mg), with *M* 1 166 (FAB m.s.). 62 Protons were counted in the ^{1}H n.m.r. spectrum and in view of the structural elements revealed in the spectrum the formula $C_{59}H_{62}N_2O_{23}$ was allocated to this compound.

The ^{1}H n.m.r. (Experimental section) showed the protons of a euonyminol core, carrying ester functions at eight hydroxy groups, and with a free tertiary 4-OH. The same 3,13 bridging dilactone observed in cathedulin-K19 was also present—^{1}H n.m.r. shifts for this segmeht are given in structure (**6**). Two acetate units, a benzoate, a nicotinate, and a (methylated) syringate (4-hydroxy-3,5-dimethoxybenzoate) complete the esterification pattern. These residues are placed by analogy with cathedulin-K19, -E3, -E5, and -E6,[4] with the syringate



(**6**)

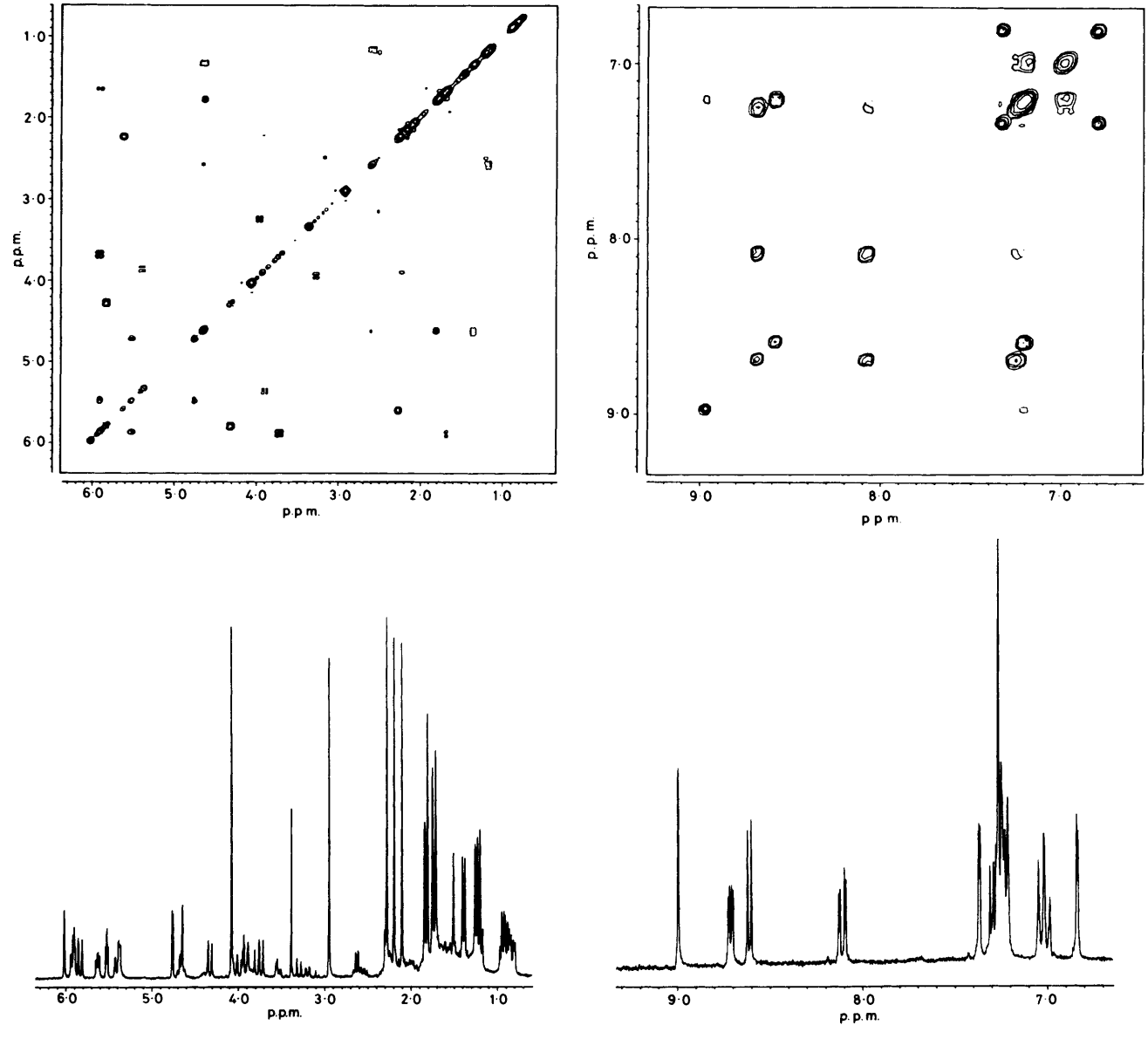


Figure 1. (a) ^{1}H–^{1}H N.m.r. spectrum (COSY 90), 0—6 p.p.m., for cathedulin-K20 (**8**). (b) ^{1}H–^{1}H N.m.r. spectrum (COSY 90), 6—10 p.p.m., for cathedulin-K20 (**8**)

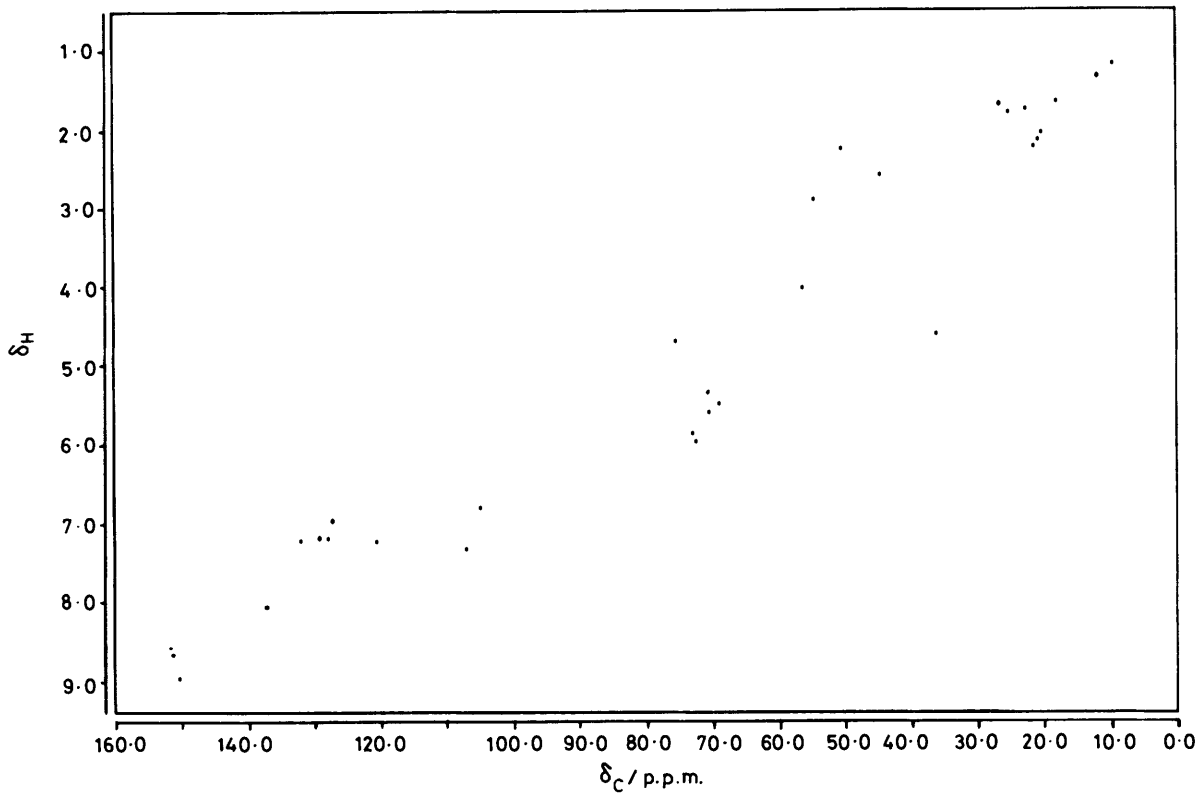
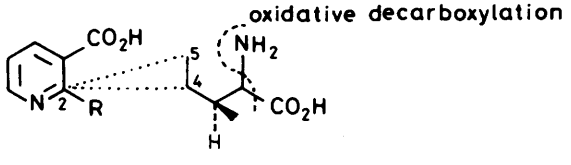


Figure 2. ^{13}C–^{1}H N.m.r. correlation spectrum for cathedulin-K20 (**8**)

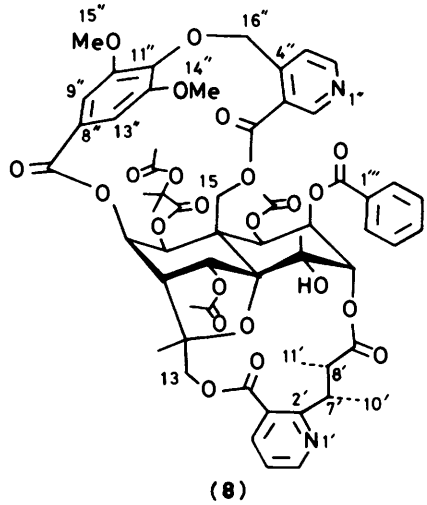
and nicotinate moieties at C-8 and C-15 respectively. This is in accord with the biosynthetic view that the cathate bridge is formed by an aryl-*O*-methyl coupling to the nicotinate nucleus. The benzoate is sited at C-2, where it replaces the shielded C-2 acetate of alkaloid-K19 (2-OCOMe, δ_H 1.34).

The new structural component (**3**) seen in both cathedulins K17 and K19 may share a similar biogenetic origin to evoninic acid (**2**) since both may be viewed as products of coupling of (at a different oxidation level) nicotinic acid (or quinolinic acid) at C-2, with C-4 or C-5 of an intermediate derived from isoleucine, as pictured in structure (**7**). In agreement, the absolute configuration at C-3 of isoleucine is the same as at C-8′ of evoninate [see structure (**8**) for numbering], and the same configuration may, on biogenetic grounds, be present in compound (**3**) although this has not been determined.



(**7**)

Alkaloid-K20 (**8**) was obtained in small quantity (11 mg), but sufficient to permit collection of ^{1}H n.m.r. spectra at 250 and 400 MHz, ^{13}C n.m.r., ^{13}C–^{1}H 2D correlation, 2D-COSY 90, and mass spectra. The formula $C_{59}H_{62}N_2O_{23}$ was indicated by $(M + H)^+$ 1 167.38 (FAB m.s.), proton integration, and counting of carbon resonances. The spectra (Experimental section) permit structure (**8**) to be assigned to cathedulin-K20. All protons could be accounted for in the ^{1}H n.m.r. spectrum, and all vicinal relationships were revealed in the COSY-90 spectrum (Figure 1). 36 Protonated carbons are present, and the correlation spectrum (Figure 2) linked 33 signals to their attached protons. C-16″, C-13, and C-15 Signals were not detected, probably because they each carry a widely separated pair of protons (H_a–H_b separation 1.51, 2.18, and 1.47 p.p.m. respectively). The ester functions are placed by analogy with



(**8**)

cathedulin-E3, with the C-2 hydroxy group esterified by benzoic acid. This is indicated by the absence of the high-field (δ_H 1.35) acetoxy methyl signal seen in the spectrum of cathedulin-E3.

Experimental

Extraction of Catha edulis.—Fresh young leaves and stems of *C. edulis* (purchased in Kenya and sent by air to Nottingham)

were freeze-dried, and powdered. A sample (1 022 g) was stirred with methanol (9 dm^3), at ambient temperature, for 48 h. The extract was evaporated to 1 dm^3, and diluted with water (1 dm^3). The solution was extracted with benzene (4 × 1.5 dm^3). The organic extracts were dried and evaporated. The residue (5.7 g) was chromatographed on a Florisil column (250 g), taking 100 cm^3 fractions. Elution commenced with benzene, and solvent polarity was increased after every ten fractions, using the following order of solvent mixtures: benzene, benzene–ethyl acetate (95:5), (90:10), (80:20), (70:30), (60:40), (50:50), then benzene–ethyl acetate–ethanol (50:50:1.5), (50:50:2.5), (50:50:5), (50:50:10), (50:50:50), (20:40:40), and finally ethyl acetate–ethanol (50:50). The fractions were monitored by t.l.c. using Dragendorff's reagent to detect alkaloids. Fractions 41—110 appeared to contain alkaloids and were evaporated. The residue (4.5 g) was chromatographed (Waters LC 500) using a Prep Pak 500 silica cartridge. Ethyl acetate–hexane (9:1) was used as eluant, at 100 cm^{-3} min^{-1}, and 14 fractions (250 cm^3) were collected. Fractions 6—14 were evaporated (1.55 g), the residue was dissolved in ethyl acetate, and a basic fraction (464 mg) was removed with dil. hydrochloric acid. This fraction was rechromatographed using h.p.l.c. [Waters 8 × 100 mm silica Rad Pak; ethyl acetate–hexane (7:3)], and the major fraction (158 mg, K' 5.5)* was repurified by reverse-phase h.p.l.c. [Waters 8 × 300 mm; C_{18}-Bondapak; methanol–water (4:1)]. The major fraction (77 mg, K' 0.88) was pure *cathedulin*-K19 (**1**). A further sample (11 mg) was obtained from an adjoining fraction. Cathedulin-K19 was an oil [Found: $(M + H)^+$, 1 103.34. $C_{54}H_{58}N_2O_{23}$ requires M, 1 102.34]. It had δ_H (250 MHz; $CDCl_3$) 9.19 (1 H, s, 2″-H), 8.83 (1 H, dd, J 2 and 5 Hz, 6′-H), 8.79 (1 H, d, J 5 Hz, 6″-H), 8.46 (1 H, dd, J 2 and 8 Hz, 4′-H), 7.68 (1 H, d, J 5 Hz, 5″-H), 7.42 (1 H, d, J 1 Hz, 9″-H), 7.42 (1 H, m, 5′-H), 6.95 (1 H, d, J 1 Hz, 13″-H), 6.74 (1 H, d, J 11 Hz, 7′-H), 6.41 (1 H, d, J 11 Hz, 16″-H_a), 5.95 (1 H, s, 6-H), 5.91 (1 H, dd, J 11.03 and 10.7 Hz, 8′-H), 5.81 (1 H, d, J 3.3 Hz, 1-H), 5.65 (1 H, dd, 8-H), 5.59 (1 H, d, J 12 Hz, 13-H_a), 5.48 (1 H, d, J 12 Hz, 15-H_a), 5.31 (1 H, d, J 5.9 Hz, 9-H), 5.10 (1 H, dd, 2-H), 4.89 (1 H, d, 16″-H_b), 4.67 (1 H, d, J 2.5 Hz, 3-H), 4.54 (1 H, br s, OH), 4.09 (3 H, s, OMe), 3.96 (1 H, d, 13-H_b), 3.91 (1 H, d, 15-H_b), 3.18 (1 H, d*q*, J 10.7 and 6.8 Hz, 9′-H), 3.10 (3 H, s, OMe), 2.21, 2.15, 2.15, and 1.34 (each 3 H, s, COMe), 1.84 and 1.75 (both 3 H, s, 12- and 14-H_3), 1.63 and 1.56 (both 3 H, s, CMe_2), and 1.24 (3 H, d, 11′-H_3); δ_C($CDCl_3$) 173.7, 171.9, 170.0, 169.8, 169.7, 168.8, 164.7, 164.4, and 157.6 (all s, 9 × C=O), 165.7 (C-2′), 154.4 and 153.8 (both s, C-10″ and 12″), 153.1 (d, C-2″), 153.0 (d, C-6′), 150.7 (d, C-6″), 145.5 (s, C-11″), 138.8 (s, C-4″), 138.1 (d, C-4′), 132.7 and 129.0 (both d, C-7′ and -8′), 129.0 (d, C-5′), 126.0, 125.8, and 124.4 (all s, C-3′, -3″, and -8″), 122.2 (d, C-5″), 106.9 and 105.9 (both d, C-9″ and -13″), 93.2 (s, C-5), 85.4 (s, C-11), 78.5 (s, C-4), 76.3, 72.8, 72.7, 71.4, 70.8, 70.0, 69.9, 69.2, and 68.5 [C-1, -2, -3, -6, -8, -9, -13, and -16″, and Me_2C(OR)CO], 62.1 (t, C-15), 56.4 and 55.1 (both q, C-14″ and -15″), 52.4 (s, C-10), 50.9 (d, C-7), 40.5 (d, C-9′), 26.9, 25.4, 23.2, and 17.8 (all q, C-12 and -14, and Me_2C), 21.5, 21.0, 20.6, and 20.4 (all q, 4 × *Me*CO), and 17.7 (q, C-11′). Assignments as CH_3, CH_2, or CH were aided by DEPT spectra.

Fractions 4 and 5 (1.5 g) from preparative h.p.l.c. were chromatographed again on the Waters LC 500 (Pre Pak 500 cartridge) using ethyl acetate–hexane (17:3) as eluant. Nine fractions (250 cm^3) were collected. Fractions 5—9 (70.0 mg in total) were rechromatographed on a 8 × 100 mm, 10μ silica Rad-Pak column with ethyl acetate–hexane (7:3) as eluant, and the fraction K' = 5.0 was re-run on the same column with ethyl acetate–hexane (3:2). Two fractions (K' = 8) were finally purified by reversed-phase h.p.l.c. (8 × 100 mm, C_{18} Rad-Pak) with methanol–water (7:3) as eluant; the fraction with K' = 11.2 gave *cathedulin*-K20 (**8**) (11 mg) [Found: $(M + H)^+$, 1 167.38. $C_{59}H_{69}N_2O_{23}$ requires M, 1 166.28]. The compound had δ_H (250 MHz; $CDCl_3$) 8.99 (1 H, s, 2″-H), 8.71 (1 H, dd, J 2.5 Hz, 6′-H), 8.61 (1 H, d, J 5 Hz, 6″-H), 8.11 (1 H, dd, J 2 and 8 Hz, 4′-H), 7.37 (1 H, d, J 1.7 Hz, 9″-H), 7.3—7.1 (5 H, 5′-, 5″-H, 2‴-, 4‴-, and 6‴-H), 7.02 (2 H, dd, J 7 and 7 Hz, 3‴- and 5‴-H), 6.84 (1 H, d, 13″-H), 6.02 (1 H, s, 6-H), 5.92 (1 H, d, 13-H_a), 5.91 (1 H, d, J 3.3 Hz, 1-H), 5.83 (1 H, d, J 11 Hz, 16″-H_a), 5.63 (1 H, dd, J 4.1 and 5.9 Hz, 8-H) 5.52 (1 H, m, 2-H), 5.39 (1 H, d, 15-H_a), 5.38 (1 H, br d, 9-H), 4.76 (1 H, d, J 2.7 Hz, 3-H), 4.66 (1 H, br q, 7′-H), 4.65 (1 H, OH), 4.32 (1 H, d, J 11 Hz, 16″-H_b), 4.08 (3 H, s, OMe), 3.91 (1 H, d, J 13 Hz, 15-H_b), 3.73 (1 H, d, J 11 Hz, 13-H_b), 2.95 (3 H, s, OMe), 2.63 (1 H, br q, 8′-H), 2.28, 2.19, and 2.10 (each 3 H, s, COMe), 1.84, 1.81, 1.75, and 1.71 (each 3 H, s, 12-and 14-H_3, and Me_2C), 1.39 (3 H, d, J 7 Hz, 10′-H_3), and 1.22 (3 H, d, J 7 Hz, 11′-H_3); δ_C (100.61 MHz; $CDCl_3$) 173.8, 171.89, 170.17, 169.75, 169.59, 168.30, 165.01, 164.06, and 163.24 (9 × CO), 165.3 (C-2′), 154.43 and 153.43 (C-10″ and -12″), 152.22 (C-6″), 151.61 (C-6′), 150.69 (C-2″), 145.21 (C-11″), *ca.* 138 (C-4 and -4″), 132.4, 128.03, 125.21, 124.82, and 124.26 (C-3′, -3″, -8″, -1‴, and -4‴), 129.63 (C-3‴ and -5‴), 127.61 (C-2‴ and -6‴), 128.44 and 121.10 (C-5′ and -5″), 107.48 and 105.35 (C-9″ and -13″), 93.65 (C-5), 84.74 (C-11), 78.27, 71.03, 69.73, and 69.34 (C-4, -13, and -16″, and Me_2COR), 75.94 (C-3), 73.37 (C-1), 72.78 (C-6), 70.77 (C-9), 70.56 (C-8), 68.36 (C-2), 61.88 (C-15), 56.66 and 54.86 (C-14″ and -15″), 51.88 (C-10), 50.68 (C-7), 44.89 (C-8′), 36.53 (C-7′), 26.86, 25.45, 23.04, 21.58, 21.04, 20.56, and 18.33 (7 × Me), 12.09 (C-11′), and 9.77 (C-10′).

In another experiment an alkaloid fraction (0.6 g) containing cathedulin-K19 (**1**) was rechromatographed on alumina, using benzene–ethyl acetate–ethanol (90:90:1) as eluant, to afford, in order of elution, cathedulin-E2 (6.5 mg) (identified by comparison with an authentic sample[2]); cathedulin-K17 (**6**) (2.5 mg); and cathedulin-K19 (**1**) (15 mg), m.p. 249—251 °C (from methanol).

Alkaloid K17 (**6**) had δ_H (250 MHz; $CDCl_3$) 8.9 (1 H, br s, 2″-H), 8.75 (1 H, dd, 6′-H), 8.60 (1 H, dd, J 2 and 5 Hz, 6″-H), 8.47 (1 H, dd, J 2 and 8 Hz, 4′-H), 7.76 (1 H, br d, J 8 Hz, 4″-H), 7.57 (2 H, d, J 7 Hz, *ortho*-PhCO–), 7.44 (1 H, dd, J 5 and 8 Hz, 5′-H), 7.4—7.0 (4 H, 5″-H, and *meta*- and *para*-PhCO), 7.14 (2 H, s, 2‴- and 6‴-H), 6.82 (1 H, J 12 Hz, 7′-H), 6.14 (1 H, s, 6-H), 5.93 (1 H, dd, J 12 and 11 Hz, 8′-H), 5.85 (1 H, d, J 2.5, 1-H), 5.70 (1 H, m, 2-H), 5.67 (1 H, d, J 11 Hz, 13-H_a), 5.57 (1 H, br dd, 8-H), 5.41 (1 H, d, J 5.5 Hz, 9-H), 5.34 (1 H, d, J 12 Hz, 15-H_a), 4.97 (1 H, d, J 3 Hz, 3-H), 4.96 (1 H, d, 15-H_b), 4.6 (1 H, br s, OH), 3.99 (1 H, d, 13-H_b), 3.85 (3 H, s, OMe), 3.83 (6 H, s, 2 × OMe), 3.28 (1 H, dq, J 7 and 11 Hz, 9′-H), 2.19, 2.06, 1.80, 1.79, and 1.74 (each 3 H, s, 3 × Ac, and 12- and 14-H_3), 1.71 and 1.64 (both 3 H, s, Me_2C), and 1.32 (3 H, d, J 7 Hz, 11′-H_3).

Acknowledgements

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* $K' = (V_1 - V_0)/V_0$ where V_1 is the sample retention volume and V_0 is the column void volume.