

The cathedulins alkaloids

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ABSTRACT

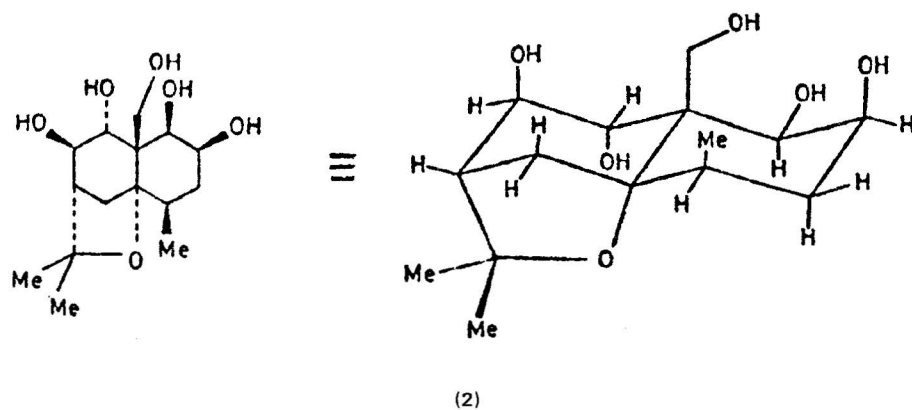
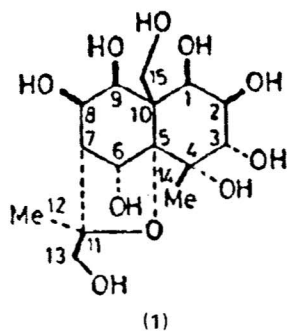
Studies on fresh and dried leaf and shoot material of *Catha edulis* (khat) collected in Ethiopia, Kenya and the Yemen Arab Republic have led to the isolation, separation and characterization of new celastraceous alkaloids, the cathedulins, with molecular weights in the 600–1,200 range. All the cathedulins whose structures have been investigated prove to be polyesters or lactones of a sesquiterpene polyol core and fall into three groups: (a) low molecular weight esters of pentahydroxydihydroagarofuran; (b) cathedulins of medium molecular weight characterized by the possession of a euonyminol core and an evoninic acid dilactone bridge; and (c) high molecular weight, more complex esters of euonyminol. Chemical evidence and spectral data were used in assigning structures to the cathedulins studied as well as in placing the various esterifying acids on the different hydroxyl positions of the sesquiterpene core. In addition to cathedulins, neutral products isolated from khat include β -sitosterol and its glycoside, friedeline, and hydroxylated $\Delta^{4\text{-exo}}$ -relatives of the latter. Moreover, the pigmented root-bark contains triterpenoid quinones including celastrol, pristimerin, iguesterin and tingenone (tingenin A and B).

As a result of collaborative work between the Chemistry Department of the University of Nottingham and the United Nations Narcotics Laboratory considerable progress has recently been made in the isolation, separation and structure determination of the group of large alkaloids of khat, the cathedulins [1–4]. Fresh leaf and young shoot material of khat was collected in Ethiopia, Kenya and the Yemen Arab Republic. Some plant material was extracted fresh, some after freeze-drying, and some after sun-drying. Various solvent-extraction procedures were employed, emphasis being laid on the content of weak bases. In one procedure leaves were treated with ammonia and then extracted with ether; treatment with ammonia increased the yield of bases without essentially altering the alkaloid composition. The bases were then isolated via dilute acid treatment and separated by chromatography. Alternatively, ethanol extraction was employed and the diluted extract brought to pH 5.5 before washing it with benzene. The neutral products were then separated chromatographically.

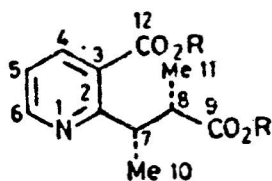
Although quantitative differences in composition between fresh and dried khat and between materials of different ages were noted, gross differences in the

numbers and types of constituent alkaloids were not observed. The composition of the alkaloid mixture was, however, dependent on the geographical source of the khat specimen. Plant material from the Yemen Arab Republic gave five alkaloids, cathedulin Y1 (identical with K1 below), Y7, Y8, Y9 and Y10, but because of the very limited supplies of material available, only Y1 could be subjected to full structural analysis. Cathedulin Y8, however, appears identical with E8, and the trace amounts of Y7, Y8 and Y10 were shown by mass spectrometry to be sesquiterpene polyol esters similar in type to other cathedulins. Kenyan khat provided pure cathedulins K1, K2, K6, K11, K12 and K15, while cathedulins E2, E3, E4, E6 and E8 were isolated from Ethiopian khat. The only alkaloid common to these two groups was E3, which was the same as K11.

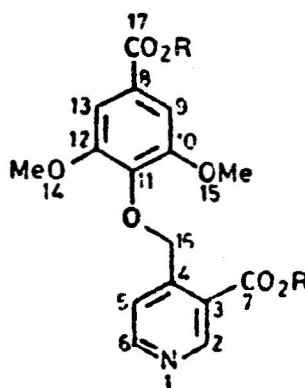
All the cathedulins whose structures have been investigated prove to be polyesters or lactones of one of the two sesquiterpene polyols (**1**) (euonyminol) and (**2**). These terpene cores are based on the eudesmane, or more specifically, agarofuran, sesquiterpene skeletons. The lower molecular weight cathedulins



E2 and E8 are relatively simple esters of the pentaol (2). Alkaloids K1, K2, K6 and K15 are of medium molecular weight and form a group based on the nonaol euonyminol (1) with one dilactone bridge. The higher molecular weight alkaloids E3, E4, E5, E6 and K12 have more complex structures based on (1) and contain various ester functions along with one or two dilactone bridges. The esterifying acid functions include acetate, benzoate, nicotinate, 2-hydroxyisobutyrate, 2-acetoxyisobutyrate and tri-*O*-methylgallate. Dilactone spans are formed from the dibasic acids (3) (evoninic) and (4) (cathic).



(3)



(4)

Some alkaloids differ from each other only in the state (free or acetylated) of certain hydroxyl functions. Thus E3, E4; E5, E6; and K1, K2, K6, K15; form sub-sets related in this way. It might be suspected that de-acetylation could be induced during extraction and chromatographic analysis, and that in consequence some of the alkaloids could be artefacts. This cannot in all cases be ruled out, though two pieces of evidence suggest that in general this is not so. Firstly, analytical thin-layer chromatography of fresh material shows essentially the same composition as the bulk extracts. Secondly, partial hydrolysis or ethanolysis of K2 leads to a set of de-acetylated products isomeric with, but different from, the natural compounds K6 and K15. The variation in alkaloid types, however, suggests that there may be a series of chemotypes of *Catha edulis*, and these aspects require further study.

The cathedulin alkaloids from khat for which structures have been proposed in the present investigation are summarized in the table.

Cathedulin alkaloids of khat

Designation	Molecular formula	Molecular weight	Melting point (°C)	Rotation (CHCl ₃)	Structure	Triterpene core	Esterifying acids ^a
E2	C ₃₈ H ₄₀ N ₂ O ₁₁	700	149–151	[α] _D ³¹ – 74°	(5)	(2)	2 Ac, 2 Nic, 1 Bzo.
E8	C ₃₂ H ₃₇ NO ₁₀	595	amorphous	—	(6)	(2)	2 Ac, 1 Nic, 1 Bzo.
K1 (Y1)	C ₄₂ H ₅₃ NO ₂₀	891	amorphous	—	(7)	(1)	6 Ac, 1 HyBu ⁱ , 1 Evon.
K2	C ₄₀ H ₅₁ NO ₁₉	849	181–184	[α] _D ²⁰ – 17.8°	(8)	(1)	5 Ac, 1 HyBu ⁱ , 1 Evon.
K6	C ₃₈ H ₄₉ NO ₁₈	807	176–180	—	(9)	(1)	4 Ac, 1 HyBu ⁱ , 1 Evon.
K15	C ₃₆ H ₄₇ NO ₁₇	765	191–194	—	(10)	(1)	3 Ac, 1 HyBu ⁱ , 1 Evon.
E3 (K11)	C ₅₄ H ₆₀ N ₂ O ₂₃	1104	245–248	[α] _D ²⁰ – 45°	(15)	(1)	4 Ac, 1 HyBu ⁱ , 1 Evon, 1 Cath.
E4	C ₅₂ H ₅₈ N ₂ O ₂₂	1062	amorphous	[α] _D ²⁷ – 37°	(16)	(1)	3 Ac, 1 HyBu ⁱ , 1 Evon, 1 Cath.
E5	C ₅₉ H ₆₄ N ₂ O ₂₃	1168	amorphous	—	(17)	(1)	3 Ac, 1 Bzo, 1 Nic, 1 MGall, 1 Evon.
E6	C ₅₇ H ₆₂ N ₂ O ₂₂	1126	amorphous	—	(18)	(1)	2 Ac, 1 Bzo, 1 Nic, 1 MGall, 1 Evon.
K12	C ₅₄ H ₆₂ N ₂ O ₂₃	1106	268–272	—	(19)	(1)	4 Ac, 1 Nic, 1 MGall, 1 Evon.

^a Ac = acetic; Nic = nicotinic; Bzo = Benzoic; HyBuⁱ = 2-hydroxyisobutyric; Evon = evoninic; Cath = Cathic; MGall = tri-*O*-methylgallic acid.

General methods of structure determination

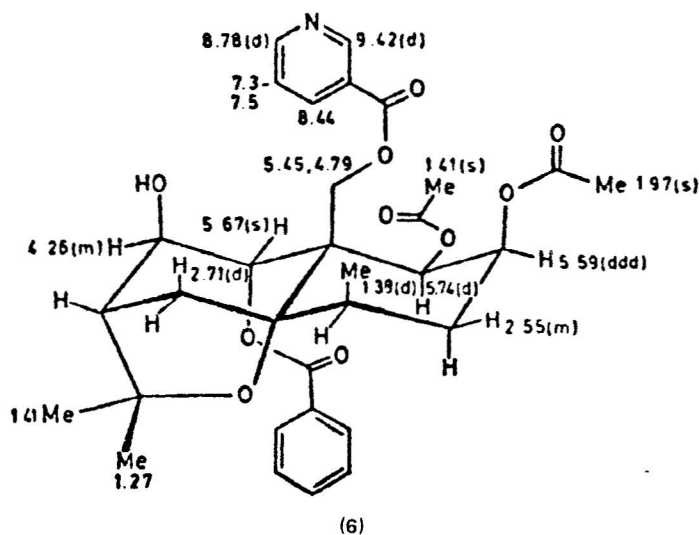
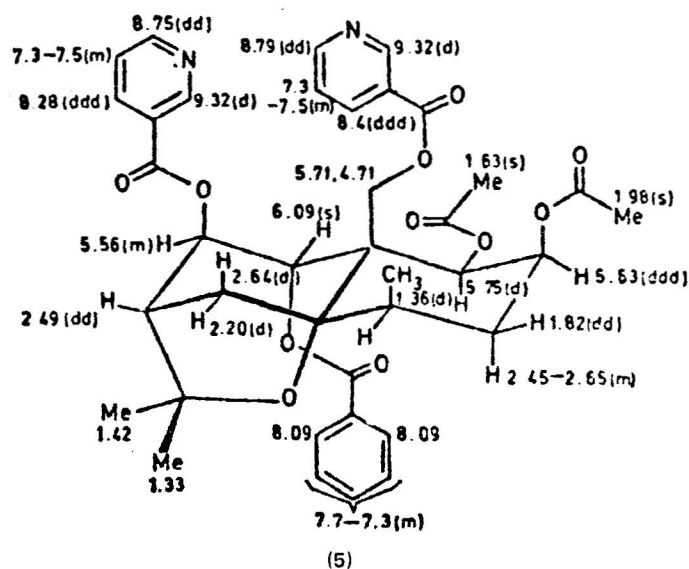
Preliminary trials of x-ray structure determination for one or two crystalline cathedulins were not satisfactory, so a combined chemical and spectroscopic approach was taken. After full spectral examination of the intact alkaloid using electron impact mass spectrometry (sometimes including chemical ionization and field desorption methods), and ^1H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy, the polyhydroxylic sesquiterpene core was isolated by alcoholysis or hydrolysis. In the case of euonyminol (**1**) the crystalline octa-acetate was prepared and compared with an authentic specimen. The core (**2**), however, was new, and its structure was established by NMR.

Esterifying acids were isolated as their esters formed by alcoholysis of the parent alkaloid. Where a single acid formed the esterifying group of more than one hydroxyl of the core, the number of molecules involved was usually evident from the NMR spectrum, but in a number of cases this was confirmed by gas-liquid chromatographic estimation methods. Of the mono-carboxylic acids encountered, 2-hydroxyisobutyric acid deserves special mention as it was previously unknown in Celastraceae alkaloids and proved initially elusive because of its high water solubility and steam volatility. An additional difficulty was the tendency of the ester to react with alkoxide ion, presumably by a $\text{B}_{\text{AL}}2$ mechanism, to give, unexpectedly, the free acid. Micromethods to deal with the problems were eventually devised. Of the dibasic acids, evoninic acid (**3**) was already known from work on other celastraceous alkaloids, but cathic acid (**4**) had not been found previously and its structure was derived from chemical and spectral studies.

The placing of the various acids on the different hydroxyl positions of the core relied on a combination of graded alcoholysis or hydrolysis studies, used to selectively strip different esterifying acids from the sesquiterpene, combined with detailed ^1H and ^{13}C NMR examinations of the original and the partially degraded alkaloids. Characteristic chemical shifts, coupling constants, specific shieldings and nuclear Overhauser effects, along with some chemical evidence, were brought to bear on the problems of drawing up the pattern of placing for the various types of esters. Sometimes chemical interconversions were helpful; thus, cathedulin E4 was converted into E3 by acetylation. The scarcity of the pure alkaloids necessitated many experiments being carried out on milligram or sub-milligram amounts of material. The graded de-esterification of cathedulin K2, which was carried out on 79 mg of alkaloid, would in comparison be classed as a large-scale experiment. This de-esterification yielded seven products, four of which were isolated in pure form.

Cathedulins of low molecular weight

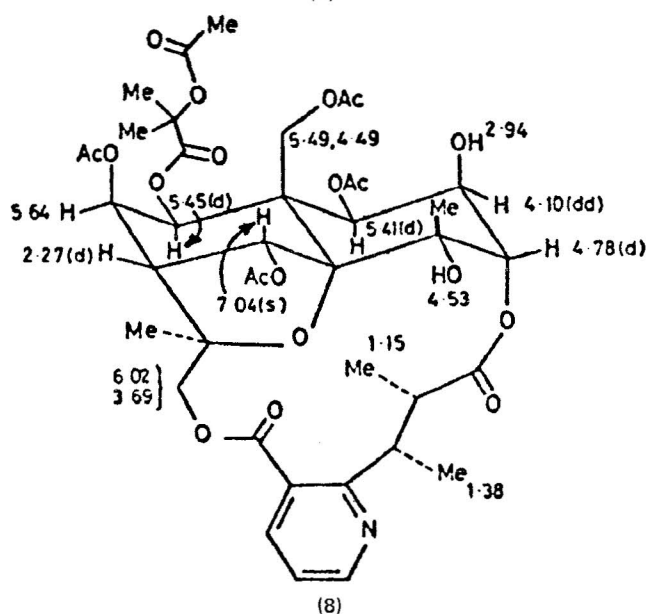
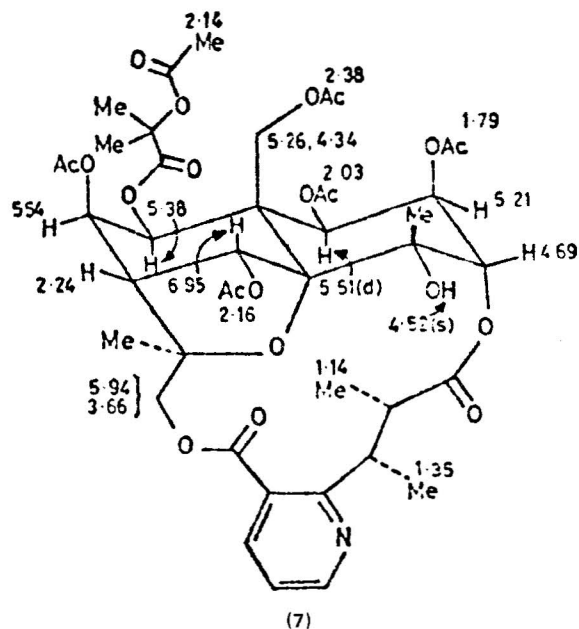
The structures proposed for cathedulin E2 and cathedulin E8 are (**5**) and (**6**). (The numbers shown on all structures are ^1H NMR assignments.) Both are based on a new 1-eq, 2-ax, 8-ax, 9-ax, 15-pentahydroxylated agarofuran-type core (**2**), which can form a cyclic carbonate bridge across the 8-ax and 15-

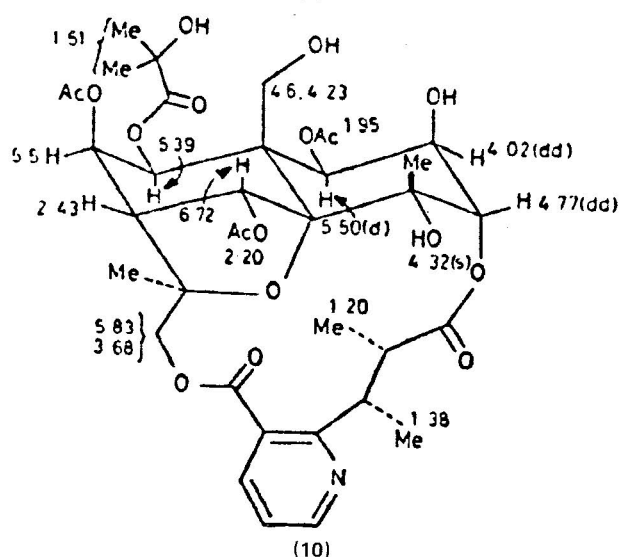
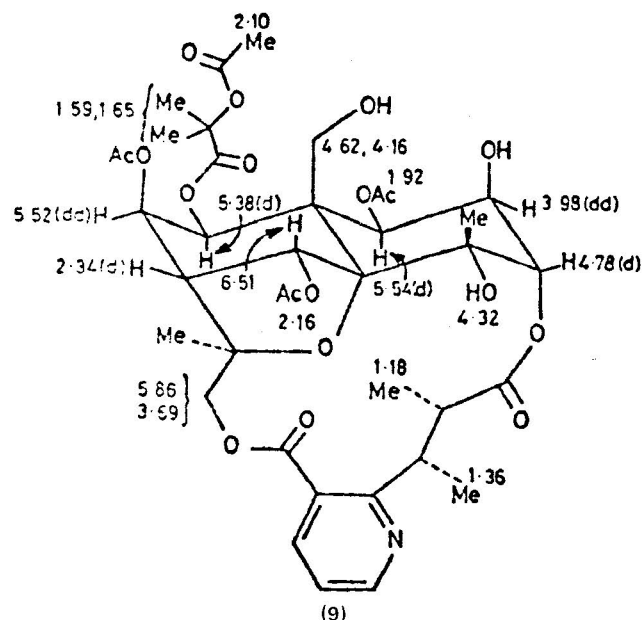


hydroxyls when treated with phosgene. The esterifying acids of cathedulin E2 are acetic (two residues), nicotinic (two residues) and benzoic (one residue) acids. Graded alcoholysis (methanolic triethylamine at 5°C) of cathedulin E2 removed the 8-nicotinoyl group giving E8. The same reagent at 25°C strips both the 8- and 15-nicotinoyl groups, while aqueous methanolic sodium bicarbonate removes the 1- and 2-acetyl moieties as well, leaving only the 9-benzoate ester. These de-esterified products showed significant changes in the proton NMR spectrum of the polyol core, which were used to assign the placing of the various esters.

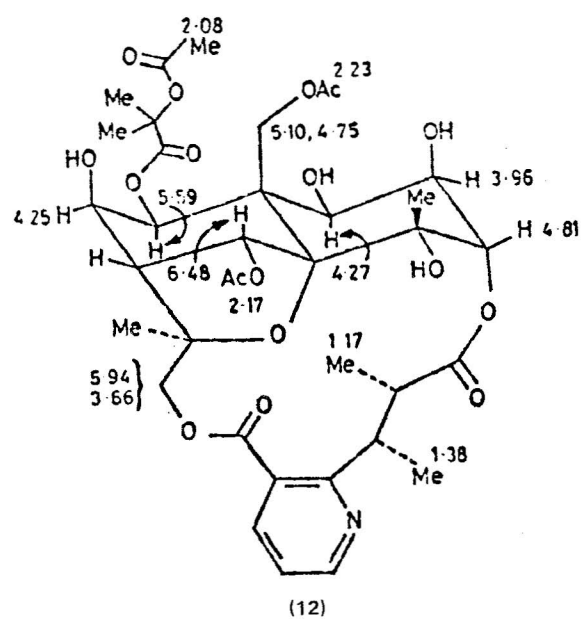
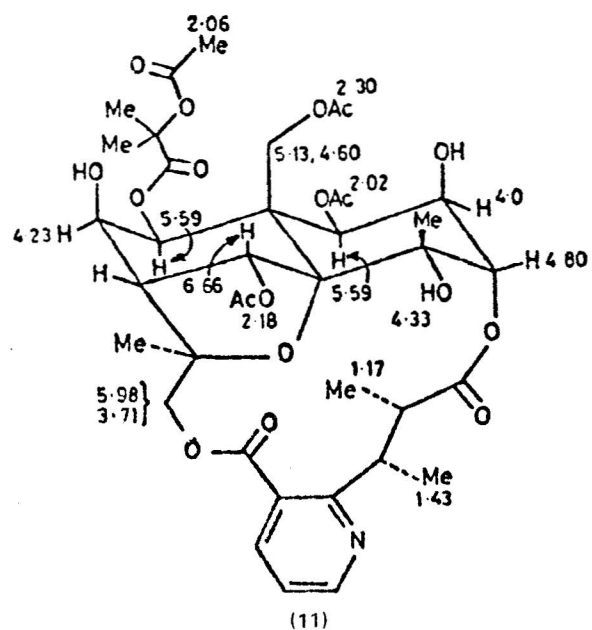
Cathedulins of medium molecular weight

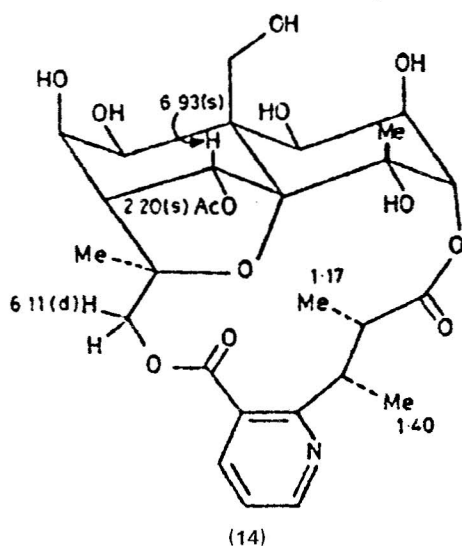
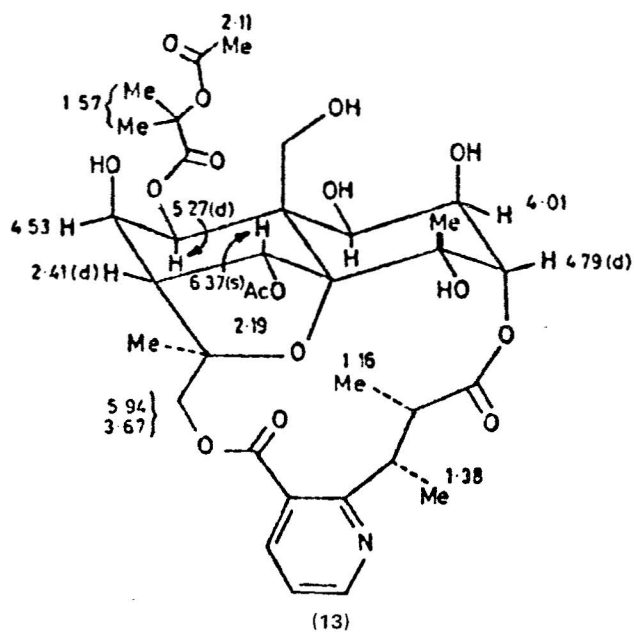
This group consists of K1, K2, K6 and K15 (structures (7), (8), (9) and (10)). Its members are characterized by the possession of a euonyminol core and an evoninic acid dilactone bridge spanning the 3–12 hydroxyl groups of the core. Their molecular weights lie in the 750–900 range.





Considerable attention was given to cathedulin K2, the esterifying acids of which comprise acetic (five residues), hydroxyisobutyric (one residue) and evoninic (one residue) acids. The latter dicarboxylic acid, and some of the others, were positioned on the basis of NMR data and the remainder were assigned to their corresponding hydroxyl groups as a result of a study of the products formed when K2 was treated with methanolic diethylamine under controlled conditions. Four partially de-esterified products were isolated, and their structures ((11), (12), (13) and (14)) were assigned on the basis of spectral data.

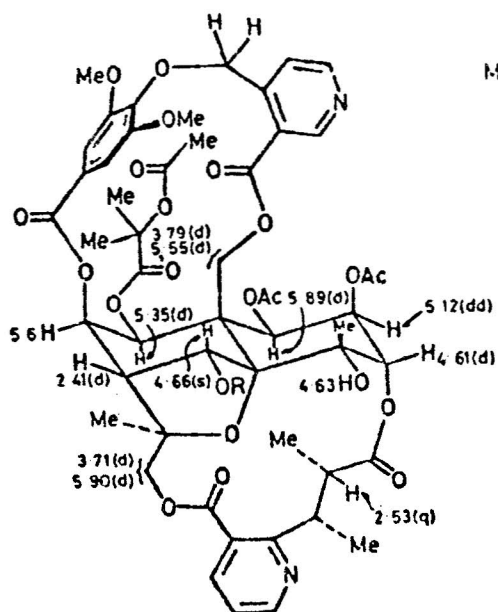




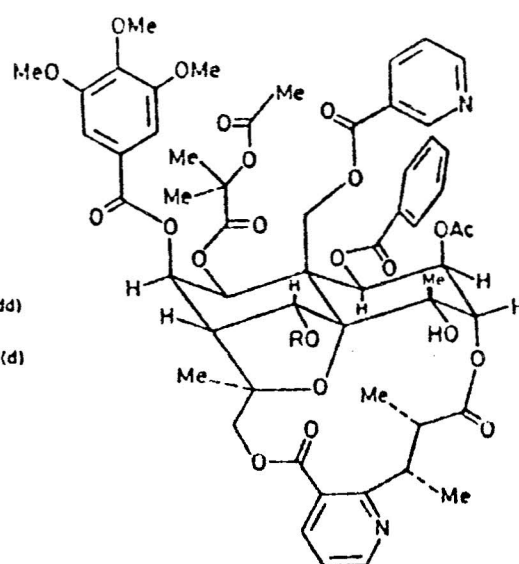
From this information on K2, together with NMR data derived from suitable models, the structures for cathedulins K1 (7), K6 (9) and K15 (10) could be arrived at.

Cathedulins of high molecular weight

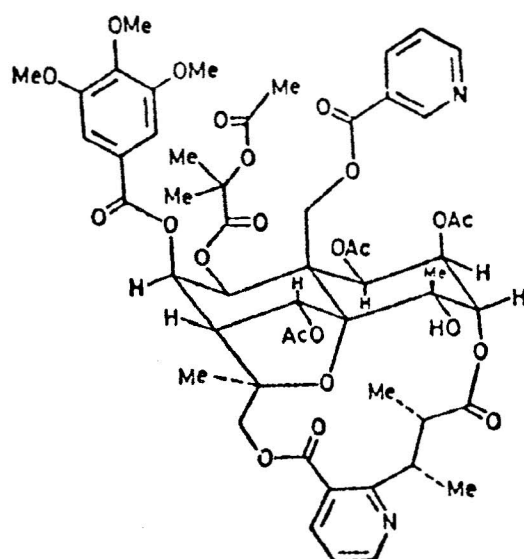
These alkaloids, E3, E4, E5, E6 and K12, were in the molecular weight range 1,000–1,200. Their structures are (15), (16), (17), (18) and (19).



(15) R = Ac
(16) R = H

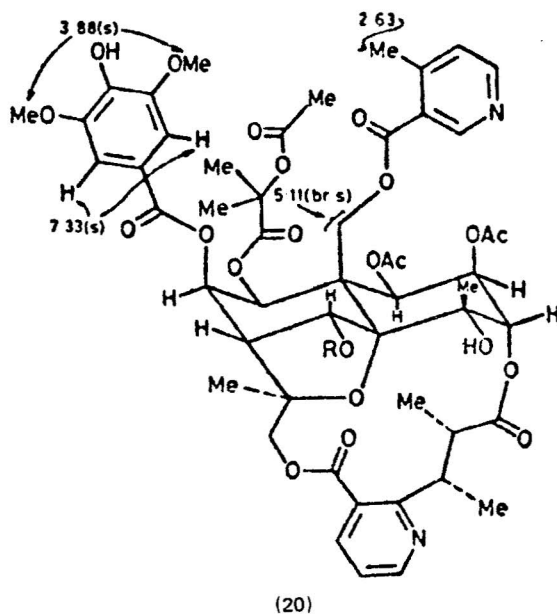


(17) R = Ac
(18) R = H



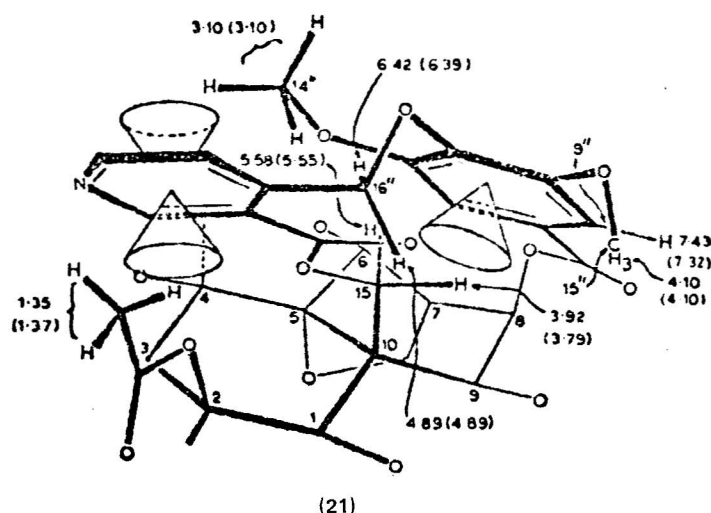
(19)

Alcoholysis (and spectral data) showed that cathedulin E3 was compiled structurally from a euonyminol core esterified by acetic (four residues), hydroxyisobutyric (one residue), and cathic (one residue) acids. On hydrogenolysis E3 underwent benzylic-type cleavage to give the *seco*-compound (20), a reaction that was accompanied by informative spectral changes. As a result of this study and ^1H and ^{13}C NMR data for the intact alkaloid, together with information from partially de-esterified products, structure (15) was proposed.



Analysis of the various shielding possibilities indicates a conformation for the cathate bridge as shown in (21) (the NMR data are for E3 and E4 (in parentheses)). The relationship between E3 and E4 is shown by acetylation of the latter which gives E3. Mass spectrometry suggests that khat contains two further alkaloids closely related to E3 and E4, but having one of the acetate esters replaced by a benzoate ester. These have not, however, been isolated in pure form.

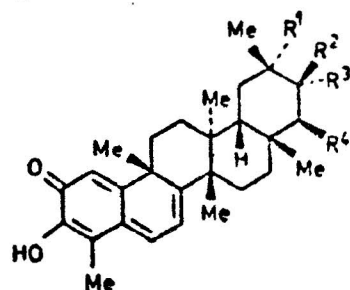
Cathedulin E6 (18) has the euonyminol core common to this sub-group and gives on methanolysis dimethyl evoninate (one residue), methyl trimethylgallate (one residue), methyl benzoate (one residue) and methyl nicotinate (one residue); one esterifying residue of 2-acetoxyisobutyric acid, together with one further acetate, was also present. Structure (18) is proposed for E6 and (17) for its monoacetylation product E5, the two alkaloids bearing the same relationship to each other as does E3 to E4. The minor alkaloid K12 (19) proved particularly interesting; although it is of the same class as E5 and E6 (because it contains both a tri-*O*-methylgallate residue and a nicotinoyl residue), it bears a special



relation to E3, from which it differs by having two additional hydrogen atoms and a seco-cathate bridge. This suggests that it could be a biosynthetic precursor of E3, which would then have the cathate span closed by cyclization between the *p*-*O*-methyl of the axial gallate residue at C-8 and the axial nicotinoyloxy-methylene residue at C-10 by a dehydrogenation reaction.

Triterpenoid components

Apart from the weakly basic cathedulin alkaloids discussed above, neutral products isolated from khat leaf and shoots included β -sitosterol and its glycoside, friedeline, and certain hydroxylated $\Delta^{4\text{-exo}}$ -relatives of the latter. Study of Ethiopian khat root showed that cathedulins E2, E3, E4, E5 and E6 were present. The red-orange pigmented root-bark contained colouring matters characteristic of the Celastraceae. These included the triterpenoid quinones celastrol (22), pristimerin (23), iguesterin (24) and tingenone, a difficultly separable mixture of tingenin A (25) and tingenin B (26).



- (22) $R^1 = \text{CO}_2\text{H}$, $R^2 = R^3 = R^4 = \text{H}$
 (23) $R^1 = \text{CO}_2\text{Me}$, $R^2 = R^3 = R^4 = \text{H}$
 (24) $R^1\text{CCR}^2 = \text{C} \equiv \text{C}$, $R^3 = R^4 = \text{H}$
 (25) $R^1 = R^4 = \text{H}$, $R^2 = R^3 = \text{O}$
 (26) $R^1 = \text{H}$, $R^2, R^3 = \text{O}$, $R^4 = \text{OH}$

References

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