residue was recrystallized from methanol: 615 mg (85%) of prisms; mp 244–246 °C dec; ^{1}H NMR (TFA) δ 3.40 (3 H, s, NCH_3), 2.95 (2 H, dd, CH_2COOH), 3.75 (1 H, m, H-4), 4.95 (1 H, br s, H-3), 5.87 (2 H, s, OCH_2O), 6.17 (2 H, s, OCH_2O), and 6.55–7.08 (5 H, m, ArH); ν_{max} (KBr) 1613, 1715, and 2400–3200 cm^{-1}.

Anal. Calcd for $C_{20}H_{17}NO_7$: C, 62.65; H, 4.47. Found: C, 62.72; H, 4.40.

***trans*-5,8-Dioxohexahydrosanguinarine (21).** A solution of 5 g (35 mmol) of P_2O_5 in 50 g of methanesulfonic acid was warmed to 45 °C. To this solution was added 500 mg (1.31 mmol) of the above acid **20**, and the mixture was stirred for 2 h while the temperature was maintained at 45 °C. The mixture was poured into ice water and extracted with chloroform. The organic solution was extracted with dilute aqueous NaOH and with water and dried, and the solvent was evaporated. The residue crystallized from ethanol: 210 mg (44%) as tan prisms; mp 277–280 °C dec; ^{1}H NMR (TFA) δ 3.38 (3 H, s, NCH_3), 2.55–4.08 (3 H, m, H-6 and H-13), 5.28 (1 H, d, $J_{13,14}$ = 11.5 Hz, H-14), 5.33 (2 H, s, OCH_2O), 5.38 (2 H, s, OCH_2O), 6.85 (1 H, d, $J_{11,12}$ = 8 Hz, H-12), 7.02 (1 H, s, H-1), 7.15 (1 H, d, $J_{11,12}$ = 8 Hz, H-11), 7.53 (1 H, s, H-4); ν_{max} ($CHCl_3$) 1640 and 1675 cm^{-1}; λ_{max} (EtOH) 213, 237, 273, and 317 nm (log ε 4.48, 4.60, 4.02, and 4.07).

Anal. Calcd for $C_{20}H_{15}NO_6$: C, 65.75; H, 4.14. Found: C, 65.71; H, 4.01.

5-Hydroxy-8-oxohexahydrosanguinarine (22). A suspension of 100 mg (0.27 mmol) of the above keto lactam **21** and 100 mg (13 mmol) of $NaBH_4$ in 100 mL of isopropyl alcohol was stirred at room temperature for 16 h. The solvent was evaporated and water added to the residue. The mixture was acidified with concentrated HCl and extracted with chloroform. The organic extracts were washed with water and dried and the solvent was evaporated. The residue crystallized from methanol: 75 mg (74%) of white prisms; mp 281–283 °C dec; ν_{max} (KBr) 1620 and 3150–3600 cm^{-1}; λ_{max} (EtOH) 219 sh, 236 sh, 290, and 318 nm (log ε 4.40, 4.17, 3.80, and 3.59).

Anal. Calcd for $C_{20}H_{17}NO_6$: C, 65.39; H, 4.66. Found: C, 65.20; H, 4.76.

Oxysanguinarine (23). A solution of 50 mg (0.14 mmol) of lactam alcohol **22** and 10 mg of *p*-toluenesulfonic acid in 50 mL of benzene was refluxed for 16 h. The solvent was evaporated and the residue was dissolved in chloroform. The solution was extracted with 5% aqueous $NaHCO_3$ and dried, and the solvent was evaporated. The residue was subjected to preparative TLC using a 3:97 methanol–chloroform solvent system. A compound with an R_f 0.62, which was significantly higher than the R_f (0.29) of the starting lactam alcohol, was obtained. Recrystallization from ether gave 15 mg (30%), mp 347–349 °C dec, spectrally and chromatographically identical with oxysanguinarine: ν_{max} ($CHCl_3$) 1645 cm^{-1}; λ_{max} (EtOH) 241, 281 sh, 289, 331, 348, 370, and 385 nm (log ε 4.27, 4.61, 4.70, 4.17, 4.18, 4.06, and 4.02).

Acknowledgments. This project was supported by NIH research grant CA-11450, awarded by the National Cancer Institute, PHS/DHEW. The assistance of a departmental grant from the NSF toward the purchase of an NMR spectrometer is also acknowledged.

Registry No.—**9**, 56920-74-2; **10**, 38699-84-2; **11**, 66271-19-0; **12**, 66271-20-3; **13**, 66303-84-2; **14**, 66271-21-4; **15**, 63254-33-1; **16**, 66271-22-5; **17**, 66271-23-6; **18**, 66303-85-3; **19**, 66271-24-7; **20**, 66271-25-8; **21**, 66271-26-9; **22**, 66271-27-0; **23**, 548-30-1; piperonal, 120-57-0; methylamine, 74-89-5.

References and Notes

(1) For reviews on this subject, see G. A. Cordell and N. R. Farnsworth, *Heterocycles*, **4**, 393 (1976); *Lloydia*, **40**, 1 (1977).
(2) M. Onda, K. Abe, and K. Yonezawa, *Chem. Pharm. Bull.*, **16**, 2005 (1968). For the revised structure of bocconine, see: H. Ishii, K. Harada, T. Ishida, E. Ueda, and K. Nakajima, *Tetrahedron Lett.*, 319 (1975).
(3) For reviews on the chemistry and synthesis of benzophenanthridines, see: M. Shamma, "The Isoquinoline Alkaloids", Academic Press, New York, N.Y., 1972, p 315; M. Shamma and J. L. Moniot, "Isoquinoline Alkaloids Research, 1972–1977", Plenum Press, New York, N.Y., 1978, in press.
(4) M. Shamma, R. W. Lagally, P. Miller, and E. F. Walker, Jr., *Tetrahedron*, **21**, 3255 (1965).
(5) R. D. Haworth, W. H. Perkin, Jr., and T. S. Stevens, *J. Chem. Soc.*, 1764 (1926).
(6) L. F. Fieser and M. Fieser, "Reagents for Organic Synthesis", Wiley, New York, N.Y., 1967, pp 892–894.
(7) D. M. Bailey, C. G. DeGrazia, H. E. Lape, R. Frering, D. Fort, and T. Skulan, *J. Med. Chem.*, **16**, 151 (1973).
(8) Reference 6, pp 880–881.
(9) W. H. Hartung and F. Crossley, "Organic Syntheses". Collect. Vol. 2, Wiley, New York, N.Y., 1943, p 363.
(10) A. F. Ferris, G. E. Johnson, and F. E. Gould, *J. Org. Chem.*, **25**, 1813 (1960).
(11) D. J. Pasto and C. R. Johnson, "Organic Structure Determination", Prentice-Hall, Englewood Cliffs, N.J., 1969, pp 183–186.
(12) P. E. Eaton, G. R. Carlson, and J. T. Lee, *J. Org. Chem.*, **38**, 4071 (1973).
(13) D. B. MacLean, D. E. F. Gracey, J. K. Saunders, R. Rodrigo, and R. H. F. Manske, *Can. J. Chem.*, **47**, 1951 (1969). For an alternate method for achieving this transformation see: A. S. Bailey and R. Robinson, *J. Chem. Soc.*, 1375 (1950); A. S. Bailey, R. Robinson, and R. S. Staunton, *ibid.*, 2277 (1950).
(14) E. Spath, F. Schlemmer, G. Schenk, and A. Gempp, *Ber. Dtsch Chem. Ges., B*, **70**, 1677 (1937).
(15) J. R. A. Pollock and R. Stevens, Ed., Dictionary of Organic Compounds", Oxford University Press, New York, N.Y., 1965, p 2188.

Chemistry of Chelocardin. 3.[1] Structure and Synthesis of Isochelocardin

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Isochelocardin (**2**), a minor component of the chelocardin fermentation, was shown to be a condensation product of two molecules of chelocardin. Carbobenzoxyisochelocardin acethydrazone (**9**) was synthesized by treatment of carbobenzoxychelocardin with chelocardin acethydrazone, thus confirming the assigned structure. The synthesis of isochelocardin itself is also described.

During the isolation of chelocardin (**1**),[2,3] a potent broad-spectrum antibiotic produced by *Nocardia sulphurea* (NRRL-2822), a contaminant which we designated as isochelocardin, was noted to be present and was subsequently isolated as a hydrochloride salt after chromatographic separation. This compound was present in the isolated chelocardin in proportions ranging from 1 to 3%. In view of the potential

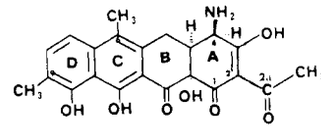

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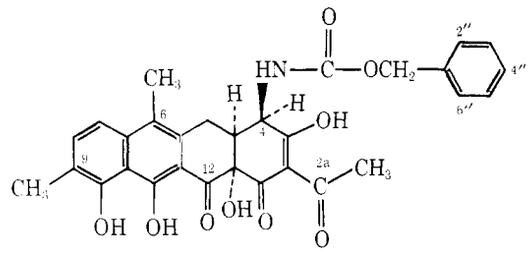
Table I. Comparative ^{13}C NMR Chemical Shift data[a] of Carbobenzoxy-β-chelocardin (4) and Carbobenzoxyisochelocardin



4

Region	4	Assignment[c]	Carbobenzoxy-isochelocardin	Region	4	Assignment[c]	Carbobenzoxy-isochelocardin
Carbonyl	200.8	12	202.7, 200.3	Aromatic	121.9	6	121.7
	200.5	2a	199.6, 199.0		119.0	9	119.3, 118.9
	196.0	1	192.5, 191.8		114.3	7	114.4, 114.3
	190.7	3	191.7, 191.3		111.2	2, 11a	111.4, 110.8
			175.1, 174.3		108.7	10a	109.0, 108.8, 108.7, 107.3, 106.8
Aromatic	162.5	11	162.9, 162.6, 162.1	Aliphatic	79.0	12a	79.8, 77.8
	157.0	C(O)N	156.7		66.1	$-CH_2Ph$	65.6
	154.7	10	154.7, 154.6		53.1	4	56.7, 55.1
	136.9	6a	136.9		41.7	4a	*b*
	136.8	1″	135.2		26.3	2a-CH_3	27.1
	135.0	8	134.9		25.6	5	26.5
	129.7	5a	130.4, 129.6, 129.3				18.1, 17.8
	128.4	2″, 6″	128.3		15.2	9-CH_3	15.2
	128.0	3″, 4″, 5″	127.8		13.7	6-CH_3	13.7

[a] Chemical shift data are given in ppm downfield from internal Me_4Si and spectra taken in Me_2SO. [b] Resonances in the aliphatic region in the carbobenzoxyisochelocardin spectrum at ~40 ppm were obscured by solvent peaks. [c] Assignments for compound **4** are taken from ref 7.

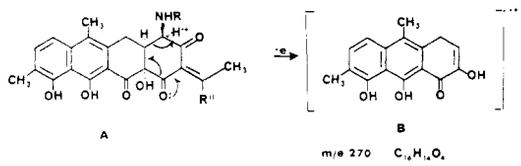
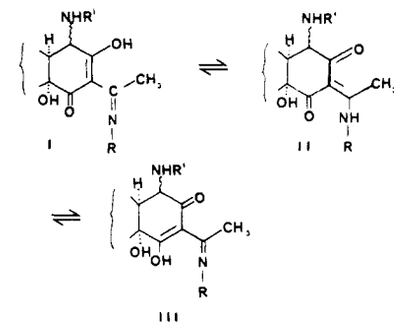


Figure 1. Major fragmentation of chelocardin and analogues.

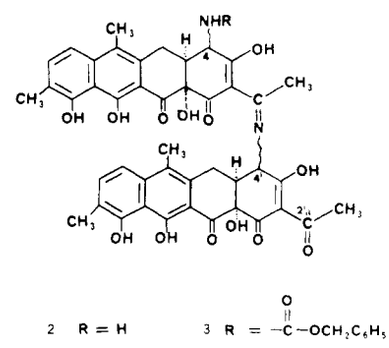
clinical use of chelocardin, the characterization of isochelocardin was of particular interest. Isochelocardin is somewhat unstable in solution at room temperature, giving rise to new impurities at a rate which depends on the nature of the solvent.

The UV absorption spectrum of isochelocardin, whose structure is shown in this report to be **2**,[8] has the characteristic chelocardin peaks[4] λ_{max}^{MeOH} 226 (ε 36 000), 273 (ε 50 400), and 438 nm (ε 9 600) and an additional absorption at 307 nm (ε 20 500). It had been observed[5] in this laboratory that 2a-substituted chelocardin analogues (in which the 2a-carbonyl group is replaced by an imine) normally possess an additional absorption at 307–312 nm. The presence of this additional absorption (307 nm) in isochelocardin suggested the possibility that it has a chelocardin skeleton with an imino substituent at the C_{2a} position.

The IR spectrum of isochelocardin shows significant difference to that of chelocardin[6] in the carbonyl region (1600–1700 cm^{-1}), but little information could be obtained from it other than an indication of the presence of a β-hydroxy-α,β-unsaturated carbonyl function or alternatively a β-amino-α,β-unsaturated carbonyl function. The ^1H NMR spectrum of this compound was of very poor resolution.[9]

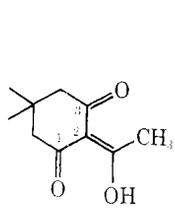


Isochelocardin formed a carbobenzoxy derivative **3** (mp 238–243 °C) upon treatment with benzyl chloroformate. The IR spectrum of **3** showed a carbamate absorption at 1730 cm^{-1}, and its ^1H NMR spectrum was poorly resolved. *N*-Carbobenzoxyisochelocardin showed a similar UV spectrum to that of the starting isochelocardin. The mass spectrum of **3** showed no molecular ion but the presence of several frag-



2 R = H 3 R = $-\overset{O}{\overset{\|}{C}}-OCH_2C_6H_5$

Table II. Some Comparative ^{13}C NMR Chemical Shift Data[a] of Carbobenzoxy-β-chelocardin and Its Analogues and 2-Substituted Dimedones



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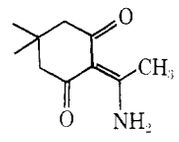

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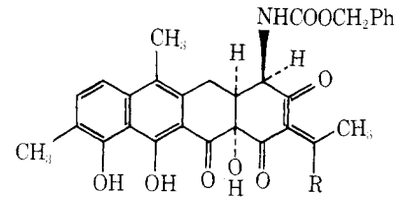


4 (R = OH), 7 (R = NH_2), 8 (R = $NHCH_2Ph$)

Assignments[c]	5	6	4	7	8
C_1	196.3	196.5	196.0	192.0	192.2
C_3	196.3	196.5	190.7	190.8	191.3
C_2	112.0	106.5	111.2	105.0	106.4
C_{2a}[b]	201.6	173.2	200.5	174.7	173.8
$C_{2a}CH_3$[b]	28	24	26.3	23.8	17.8

[a] Chemical shift data are given in ppm downfield from internal Me_4Si and spectra taken in Me_2SO. [b] Substantial upfield shift by substitution at C_{2a} carbonyl. [c] Assignments were taken from ref 7.

ments normally found in the mass spectra of chelocardin analogues; the most important and prominent ion can be assigned to structure B shown in Figure 1 (*m/e* 270, $C_{16}H_{14}O_6$)[4] confirming our previous observation that isochelocardin contains the basic chelocardin skeleton.

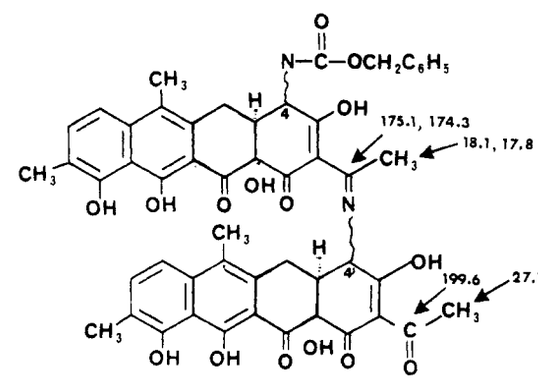
Carbobenzoxyisochelocardin (**3**) was subjected to detailed ^{13}C NMR analysis. The chemical shift data along with those of carbobenzoxychelocardin (**4**)[7] are presented in Table I. The remarkable feature of the ^{13}C NMR spectrum of compound **3** is that in many cases there are two or more resonances for each carbon of compound **4**. This suggested that carbobenzoxyisochelocardin was a mixture of two isomers in approximately equal proportions and/or that its molecular structure incorporated two molecules of chelocardin.

The ^{13}C NMR chemcal shifts[7] of the three carbonyl carbons of the β-triketone system and also the 2a-methyl of chelocardin are profoundly affected by substitution on the 2a-carbonyl, as illustrated in Table II. In each case, the C_{2a} and C_{2a}-methyl carbon resonances undergo substantial upfield shifts.

These changes are extremely useful in the structural determination of isochelocardin. In addition to our previous observation from UV and ^{13}C NMR spectra, the presence of resonance at 175.1 and 174.3 as well as at 18.1 and 17.8 ppm together with resonances at 199.6, 199.0, and 27.1 ppm in the ^{13}C NMR spectrum of carbobenzoxyisochelocardin (see Figure 2) suggested that isochelocardin has a "dimeric" structure formed by a Schiff base condensation of two molecules of chelocardin with the loss of a molecule of water. Carbobenzoxyisochelocardin would then be represented by **3**, a structure consistent with its elemental analysis. The presence of signals in its mass spectrum at *m/e* 503 and 435 is also consistent with structure **3** in that they can arise from fragmentation as outlined in Figure 3.

To confirm that carbobenzoxyisochelocardin was indeed **3** and not just a mixture of two isomers of a mono-2′-substituted carbobenzoxychelocardin, carbobenzoxyisochelocardin was converted to its acethydrazone **9** by reaction with acethydrazide in tetrahydrofuran (Scheme I), by analogy to the preparation of hydrazones from chelocardin and carbobenzoxy-chelocardin.[5] The formation of compound **9** established the presence of a free β-tricarbonyl system in the A ring of carbobenzoxyisochelocardin. The ^{13}C NMR (see Table III) and UV data for **9** are consistent with hydrazone substitution in the 2a position of a chelocardin moiety.

The structure of carbobenzoxyisochelocardin acethydrazone (**9**) was confirmed by synthesis. Treatment of carbobenzoxychelocardin with chelocardin acethydrazone hydrochloride (**10**)[5] in dimethylformamide in the presence of sodium bicarbonate gave, after purification, a clean product having the same R_f as compound **9** on TLC analysis. This product was made by reacting two distinct compounds in more than 70% yield with gradual disappearance of both starting materials; it is therefore unlikely that it was a rearrangement product of either one of the starting materials, as in that case



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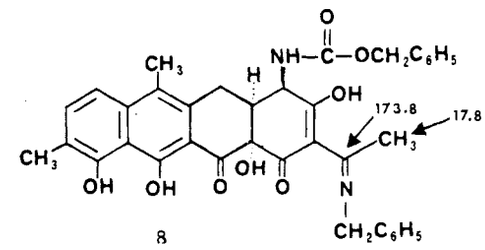

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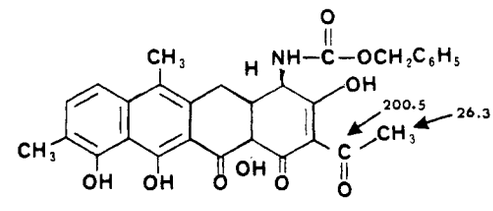

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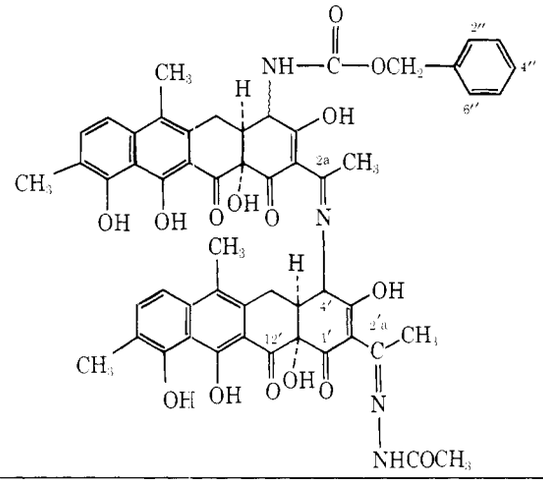
Figure 2. Some important ^{13}C NMR chemical shifts (in ppm downfield from internal Me_4Si) of carbobenzoxychelocardin derivatives as taken from ref 6.

Table III. Comparative ^{13}C NMR Chemical Shift Data[a-c] of Carbobenzoxyisochelocardin Acethydrazone (9) and Its Synthetic Counterpart (11)



11	9	Assignment	11	9	Assignment	11	9	Assignment
202.3	202.2	12, 12′	136.8	136.8	6a	107.2	107.2	2
200.7	200.0		134.8 × 2	134.7 × 2	8, 8′	104.2	104.2	2′
192.6	193.1*	1, 1′	130.4	130.4	5a, 5′a	79.7	79.7	12a
191.2	191.2		129.0	129.1	4″	78.8*	78.8*	($CHCl_3$ trace)
189.7	189.8	3, 3′	128.3 × 2	128.3 × 2	2″, 6″	78.5	78.4	12′a
188.7	188.7		127.8 × 2	127.8 × 2	3″, 5″	65.7	65.7	-CH_2Ph
174.9	174.9	2a	121.4	121.3*	6, 6′	57.5*	57.6*	4, 4′
169.3	169.3	2′a	121.2	121.2		54.9*	54.9*	
167.1	167.2	NHC(O)CH_3	118.9	118.7 × 2	9, 9′	25.8*	25.8*	5, 5′
162.8	163.2*	11, 11′	118.7			25.1*	24.8*	
162.3	162.7		114.2 × 2	114.2 × 2	7, 7′	20.2	20.2	2′a-CH_3
157.0	157.0	NHC(O)O	111.4	111.6	11a, 11′a	18.3*	18.4	2a-CH_3
	155.2	10, 10′		111.5		16.9	16.9	-NH-C(O)CH_3
154.7	154.8		108.9	108.9	10a, 10′a	15.1 × 2	15.1 × 2	9-CH_3, 9′-CH_3
136.9 × 2	136.9 × 2	1″, 6′a	108.6	108.6		13.6 × 2	13.6 × 2	6-CH_3, 6′-CH_3

[a] Chemical shift data are given in ppm downfield from internal Me_4Si and spectra taken in Me_2SO. [b] Shifts marked * were not generated by the computer but were hand calculated and are less precise; those marked ×2 are of double intensity, although in some cases the peaks are not twice as tall but reather broadened. [c] Resonances in aliphatic regions around 40 ppm were obscured by solvent.

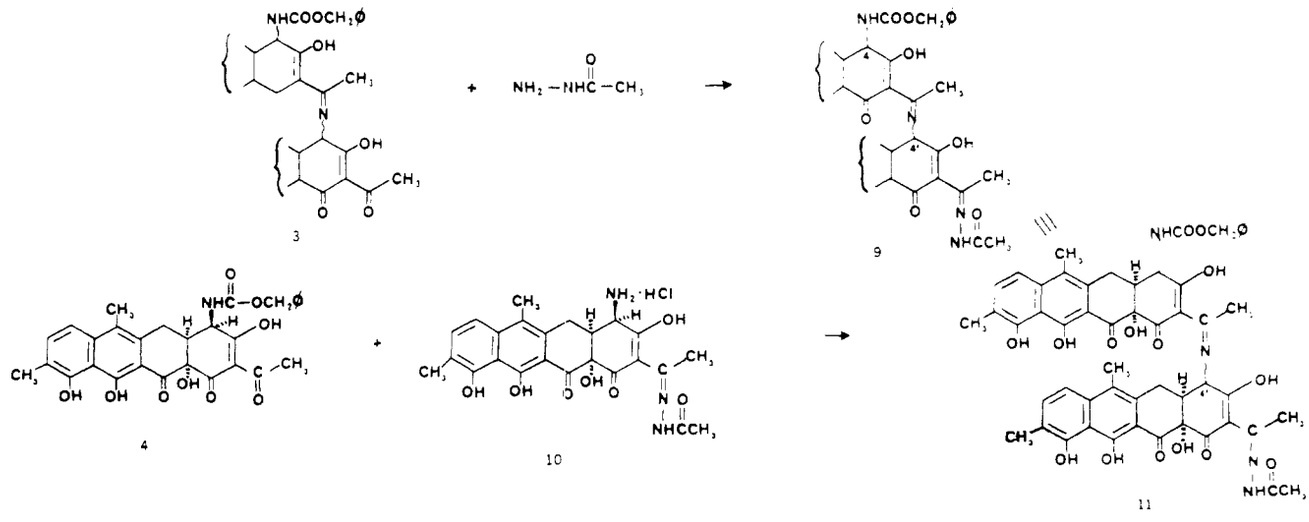
one would expect to have no more than 50% conversion. Furthermore, neither of the starting materials alone gave the product under the same experimental conditions.

The product must therefore be the expected condensation product, the Schiff base **11**. Its elemental analysis showed a nitrogen value which is compatible with a "dimeric" structure and its structure was further confirmed by ^{13}C NMR analysis (Table III).

Compound **11** appeared as one spot on TLC and appeared to be identical to compound **9**, having the same R_f in TLC, identical elemental analysis, ^{13}C NMR, and UV spectra, and very similar IR spectra. Thus, compounds **9** and **11** are unambiguously identical.

Under the same experimental conditions used to prepare compound **11**, a considerable amount of chelocardin acethydrazone (**10**) epimerized to its α epimer at C_4 (**12**). Hence,

Scheme I



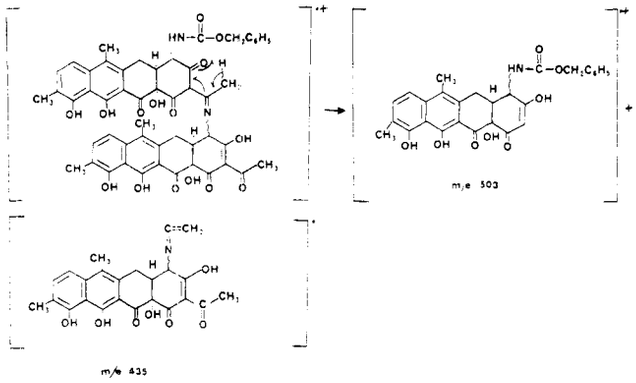
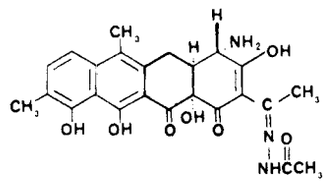


Figure 3. Initial fragmentation of carbobenzoxyisochelocardin **3**.

compound **11** as well as **9** should be a mixture of components isomeric at $C_{4'}$. This possibility was confirmed by LC. LC analysis indicated that compound **11** was a mixture of two components in a ratio of 2:1. Compound **9** has the same re-



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tention volumes as compound **11** (the ratio of the two components was 95:5). (The presence of additional isomers differing at the C_4 atom cannot be excluded, since it is possible that the LC conditions we employed did not resolve the epimer at C_4.)

Since the structure of carbobenzoxyisochelocardin acethydrazone (**11**) is confirmed, the structure of isochelocardin is fully established to be **2**, having a mixture of at least two epimers presumably at $C_{4'}$.

Isochelocardin (**2**) was synthesized by reacting chelocardin hydrochloride with a 1 molar equiv of chelocardin free base in THF to give a 65% conversion to a mixture of products with a major component having the same R_f in three different TLC systems and identical retention volumes in LC analysis as isochelocardin.

Experimental Section

Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. The infrared spectra were recorded on a Beckman Model IR8 infrared spectrophotometer. The ^1H NMR spectra were recorded on Varian Associates EM-360 and HA-100 spectrometers in deuterated solvents; resonance positions are given on the δ scale (ppm) relative to internal tetramethylsilane. The mass spectra were recorded on an AEI MS-902 double-focussing mass spectrometer. The UV spectra were recorded on a Unicam SP-800A spectrometer in 0.1 N methanolic hydrogen chloride solution. The ^{13}C NMR spectra were recorded on a Varian Associates XL-100-15/TT-100 spectrometer system in Me_2SO; resonance positions are give in ppm relative to internal tetramethylsilane. Parameters used were pulse width (30°) 3.5 μs, pulse delay 0.5–1.0 s, 6K sweep width, and 8K data table. LC analyses were performed on a Waters Associates ALC-202 instrument through a phenyl/corasil column (1/8 in. × 24 in.). Detection was by UV absorbance at 280 nm. Injections were performed with a Waters Model U6K injector.

The IR absorption spectrum of isochelocardin hydrochloride is uncharacteristic. Since the various derivatives reported here have a β-hydroxy-α,β-unsaturated carbonyl function which would have a similar absorption to the β-diketone system, very little change in the carbonyl absorption region (1580–1680 cm^{-1}) was observed. However, the changes in relative intensity of the carbonyl absorptions correlated with the structural changes. Thus, only the relevant difference in absorption bands will be mentioned in the Experimental Section. Since all the ^1H NMR spectra obtained are of poor resolution, they are not reported. The relevant ^{13}C NMR data are given above.

The abbreviations used both in the text and in the Experimental Section are designated as follow: TLC, thin layer chromatography; DMF, dimethylformamide; THF, tetrahydrofuran; IR, infrared; UV, ultraviolet; ^1H NMR, proton magnetic resonance; ^{13}C NMR, carbon magnetic resonance; LC high pressure liquid chromatography.

Isolation of Isochelocardin Hydrochloride (2). Crude chelocardin–calcium chloride complex from fermentation sources having 2% of isochelocardin was chromatographed on Sephadex (LH-20) using a 0.1 N methanolic hydrogen chloride solution as the eluting solvent. The isochelocardin hydrochloride was isolated as a deep orange amorphous solid: UV λ_{max}^{MeOH} 226 (ϵ 36 000), 273 (ϵ 50 400), 307 (ϵ 20 500), and 438 nm (ϵ 9 600).

Carbobenzoxyisochelocardin (3). Sodium bicarbonate (168 mg; 2 mM) and benzyl chloroformate (120 mg; 0.67 mM) were added to a solution of isochelocardin **2** (200 mg; 0.45 mM) in 50 mL of 96% aqueous THF. The reaction mixture was stirred for 1 h at room temperature. Water (45 mL) was added and the THF was evaporated under reduced pressure. The aqueous suspension was extracted with ether. After decanting the ether, the aqueous layer was acidified to pH 1–2 with 5% hydrochloric acid and extracted with chloroform (2 × 80 mL). The chloroform extract was washed twice with water, dried, and evaporated to dryness. The residue was washed with ether to give 192 mg of product **3**: mp 238–243 °C; UV λ_{max}^{MeOH} 224 (ϵ 50 000), 273 (ϵ 77 000), 300 (ϵ 33 000), 311 (ϵ 30 000), and 432 nm (ϵ 11 000); the IR spectrum showed a carbamate absorption at 1730 cm^{-1}; MS *m/e* 503, 435, 394, 270, 255, 109, and 91. Anal. Calcd for $C_{52}H_{46}N_2O_{15}$: C, 66.50; H, 4.90; N, 2.99; O, 25.60. Found: C, 66.73; H. 5.09; N, 2.91; O, 25.25.

Carbobenzoxyisochelocardin Acethydrazone (9). Acethydrazone (24.5 mg; 0.33 mM) was added to a solution of carbobenzoxyisochelocardin (**3**) (180 mg; 0.33 mM) in 25 mL of THF and the mixture was stirred at room temperature for 1 h. The solution was then evaporated to dryness under reduced pressure, taken up in chloroform (3 mL), and precipitated with methanol. Upon filtration, a dark yellow solid was obtained, which was then purified by preparative thin layer chromatography on Quanta-gram precoated silica plates (eluting solvent system: chloroform–methanol–acetic acid (20:1:1 v/v aged for 24 h)), yielding compound **9** (145 mg): UV λ_{max}^{MeOH} 224 (ϵ 49 000), 272 (ϵ 80 000), 300 (ϵ 43 300), 311 (ϵ 43 300), and 430 nm (ϵ 11 900). Anal. Calcd for $C_{54}H_{50}N_4O_{15}$: C, 64.18; H, 5.06; N, 5.63; O, 24.12. Found: C, 64.50; H, 5.00; N, 5.37; O, 24.90.

Condensation between Carbobenzoxychelocardin and Chelocardin Acethydrazone (Synthetic Carbobenzoxyisochelocardin Acethydrazone (11)). Carbobenzoxychelocardin[6] (545 mg; 1 mM) and chelocardin acethydrazone hydrochloride[5] (502 mg; 1 mM) were dissolved in 12 mL of DMF. Sodium bicarbonate (84 mg; 1 mM) was added and the mixture was stirred for 5 days at room temperature. It was then added dropwise to 500 mL of ether and filtered. After purification by preparative thin layer chromatography on Quantagram Q. silica precoated plates (eluting solvent system: chloroform–methanol–acetic acid (20:1:1)) a 730 mg (72%) yield of **11** was obtained: UV λ_{max}^{MeOH} 224 (ϵ 51 000), 272 (ϵ 84 000), 300 (ϵ 45 000), 311 (ϵ 45 000), and 430 nm (ϵ 12 000). Anal. Calcd for $C_{54}H_{50}N_4O_{15}$: C, 64.18; H, 5.06; N, 5.63; O, 24.12. Found: C, 65.00; H, 5.20; N, 5.55; O, 24.80.

LC Determinations of Compounds 9 and 11. With a flow rate of 2 mL/min and using a step change procedure, starting with a mixture containing 17.50% (v/v) of acetonitrile in a 0.01 M Na_2ETDA aqueous solution adjusted to pH 8.8 and changing 5 min after injection to a mixture containing 17.43% (v/v) of acetonitrile, compound **9** was shown to have two components in the ratio of 5:95 with retention volumes of 22 and 30 mL, respectively. Under identical conditions, compound **11** was also shown to be a mixture of two components in the ratio of 1:2 with retention volumes of 22 and 30 mL, respectively.

Preparation of Isochelocardin (by Synthesis). Chelocardin hydrochloride (223 mg; 0.5 mM) was added to a solution of chelocardin free base (205 mg; 0.5 mM) in tetrahydrofuran. After stirring for a period of 15 h, a mixture of several compounds was observed. The major component had a R_f identical to isochelocardin (**2**) in three different TLC systems: (1) Quanta-gram silica gel plates deactivated with 10% oxalic acid in methanol and developed with chloroform–methanol–formic acid (90:10:5); (2) Merck aluminum precoated silica gel plates oxalic acid deactivated and developed with chloroform–methanol–formic acid (80:20:5); and (3) Merck aluminum precoated polyamide **11** plates developed with Chloroform–methanol–formic acid (90:10:5). After performing a gel filtration through Sephadex LH-20, the mixture was subjected to LC analysis using 10% acetoni-

trile in 0.01 M Na_2ETDA solvent at pH 7.8 (flow rate 2 mL/min) and the major component was found to be identical to isochelocardin according to retention volume (26 mL). The major component could not be isolated in pure enough form for full characterization.

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Registry No.—**1**, 29144-42-1; 1 HCl, 56433-46-6; **2**, 66290-79-7; **2** HCl, 66290-80-0; **3**, 66290-81-1; **4**, 65805-84-7; **9**, 66290-82-2; **10**, 66290-83-3; acethydrazone, 1068-57-1.

References and Notes

(1) For Part II, see D. T. W. Chu, S. N. Huckin, and E. Bernstein, *Can. J. Chem.*, **55**, 3341 (1977).
(2) T. J. Oliver, J. F. Prokop, R. R. Bower, and R. H. Otto, *Antimicrob. Agents Chemother*, 583 (1962).
(3) A. C. Sinclair, J. R. Schenck, G. G. Post, E. V. Cardinal, S. Burokas, and H. H. Fricke, *Antimicrob. Agents Chemother.*, 592 (1962).
(4) L. A. Mitscher, J. V. Juvarkar, W. Rosenbrook, Jr., W. W. Andres, J. Schenck, and R. S. Egan, *J. Am. Chem. Soc.*, **92**, 6070 (1970); L. A. Mitscher, W. Rosenbrook, Jr., W. W. Andres, R. S. Egan, J. Schenck, and J. V. Juvarkar, *Antimicrob. Agents Chemother.*, 38 (1970).
(5) D. L. Garmaise, D. T. W. Chu, E. Bernstein, and M. Inaba, manuscript in preparation.
(6) D. T. W. Chu, D. L. Garmaise, and E. Bernstein, *Can. J. Chem.*, **53**, 1434 (1975).
(7) R. S. Egan, R. S. Stanaszek, E. Bernstein, D. T. W. Chu, and S. N. Huckin, manuscript in preparation.
(8) In this paper structures are given as tautomer (I) for simplicity but an equilibrium with the other tautomeric forms II and III is not excluded.
(9) It has been observed[4] that the ^1H NMR spectra of chelocardin and its derivatives (other than 4-*N*-acyl derivatives) are poorly resolved.

Synthesis and Mass Spectrometry of Some Structurally Related Nicotinoids

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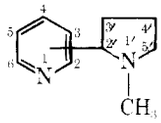
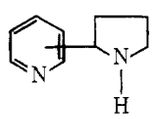
The synthesis and mass spectrometry of a group of structurally related nicotinoids (**1a–c–6a–c**) have been investigated. A detailed discussion is presented of their complex electron-induced fragmentation mechanisms, established with the aid of 27 site-labeled deuterium analogues, high-resolution measurements, and metastable ion studies.

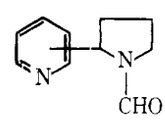
Substantial interest in the minor tobacco alkaloids, their mammalian metabolites, and the physiological effects of nicotine has been noted in the recent literature.[2–4] Further, the widespread occurrence of nicotine-like compounds in nature,[5,6] as well as the expanding interest in the trace components of tobacco and tobacco smoke,[7] have led us to undertake an investigation of the preparation and spectral properties of a group of structurally related nicotinoids (**1a–c–6a–c**). The



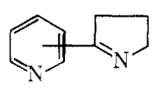
1a–c



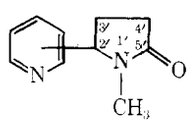
2a–c



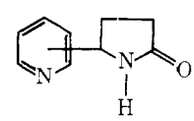
3a–c



4a–c



5a–c



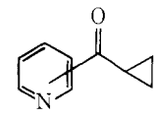
6a–c

a = 2-pyridyl; b = 3-pyridyl; c = 4-pyridyl

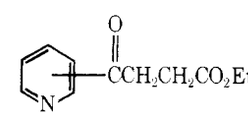
results of our synthetic and mass spectrometric studies are reported here.

Synthesis.[8] Despite the extensive studies[2–6,9–11] reported on the *Nicotiana* alkaloids (**1b–4b**), their metabolites (**5b** and **6b**), and a host of analogues, only a limited amount of diffuse work has been carried out on the isomeric nicotinoids (**1a,c–6a,c**).[12] It therefore seemed appropriate to investigate the applicability of newer methods of alkaloid synthesis to the preparation of this cohesive group of structurally related, isomeric nicotinoids.

The reaction of cyclopropyl 3-pyridyl ketone (**7b**) with formamide has been shown to give 3-nornicotine[13] (**2b**; via acid



7a–c



8a–c

a = 2-pyridyl; b = 3-pyridyl; c = 4-pyridyl

hydrolysis of *N'*-formyl-3-nornicotine (**3b**), generated in situ). This method was chosen as a route to the nornicotines (**2a** and **2c**) and the *N'*-formylnornicotines (**3a–c**). After having first established that **3b** could be isolated from the reaction of **7b** with formamide, the synthesis was used to prepare 2- and 4-nornicotine (**2a** and **2c**) and *N'*-formyl-2- and *N'*-formyl-4-nornicotine (**3a** and **3c**) from the appropriate cyclopropyl pyridyl ketone (**7a,c**).

The cotinines (**5a** and **5c**) were prepared by treatment of the pyridoylpropionates (**8a** and **8c**) with *N*-methylformamide using an altered version of Sugasawa's method.[14] The prerequisite α-keto esters were obtained from the reaction of ethyl acrylate with 2- and 4-pyridinecarboxaldehyde,[15] a method which was found preferable to the published procedure.[16]

Attempts to apply the cotinine synthesis to the preparation of the norcotinines (**6a** and **6c**) by substituting formamide for *N*-methylformamide were unsuccessful. However, **6a** and **6c** could be obtained by reaction of the appropriate α-keto ester (**8a** or **8c**) with NH_4Cl and $NaBH_3CN$.

During the course of our study, Hu[18] reported an excellent improvement of Späth's original 3-myosmine (**4b**) synthesis.[19] This method,[18] with some modification, was used to prepare the myosmines (**4a–c**).

The synthesis of the deuterated nicotinoids (Table I), necessary for both this and NMR studies,[20] proved facile ex-

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