Sporidesmins. Part XIII.[1] Ovine Ill-thrift in Nova Scotia. Part III.[2] The Characterisation of Chetomin a Toxic Metabolite of *Chaetomium cochliodes* and *Chaetomium globosum*

By **S. Safe** and **A. Taylor,*** National Research Council of Canada, Atlantic Regional Laboratory, Halifax, Nova Scotia

Chetomin, one of the antibiotic metabolites of *Chaetomium cochliodes* and *C. globosum* has been isolated as an amorphous, glass-like solid. Elemental analysis of chetomin, its diacetate, and its bistrimethylsilyl ether, mass spectroscopy of its tetrathiomethyl derivative, and n.m.r. spectroscopy indicate the molecular formula to be $C_{31}H_{30}N_6O_6S_4$. The formation of a tetra-*S*-methyl derivative and a bismonosulphide indicated the presence of two epidithiodioxopiperazine ring systems like those present in chaetocin, a metabolite of *C. minutum*, a conclusion supported by the close similarity of the c.d. of the two metabolites. The u.v. spectrum of chetomin was the same as that of echinulin. These facts, and an interpretation of the n.m.r. spectra of chetomin and its derivatives, lead us to believe that the molecule contains an indole, an indoline, and two epidithiodioxopiperazine systems. A biogenetically plausible assembly of these structural features is shown [formula (XIII)], but other formulae are possible.

In 1944 Waksman and Bugie [3] isolated a strain of *Chaetomium cochliodes* which, in culture, produced antibacterial metabolites for which they suggested the name chaetomin. They were able to extract these metabolites from the cultures [4] and later Geiger [5] obtained material (Found: C, 51·4; H, 4·2; N, 11·1; S, 16·8%) which had $[\alpha]_D$ +360° and a u.v. spectrum similar to that of skatole. No further chemical investigations on this material have

[1] Part XII, E. Francis, R. Rahman, S. Safe, and A. Taylor, preceding paper.
[2] Part II, D. Brewer, A. Taylor, and M. M. Hoehn, *J. Agric. Sci.*, in the press.
[3] S. A. Waksman and E. Bugie, *J. Bacteriol.*, 1944, **48**, 527.
[4] W. B. Geiger, J. E. Conn, and S. A. Waksman, *J. Bacteriol.*, 1944, **48**, 531.
[5] W. B. Geiger, *Arch. Biochem.*, 1949, **21**, 125.

since been published but the high antibacterial activity has been confirmed,[6] the cross resistance of bacteria to gliotoxin, sporidesmin, and chetomin has been reported,[7] and chetomin has been shown to inhibit specifically viral ribonucleic acid synthesis in a number of cell lines.[8] These biological results strongly suggest that chetomin is a member of the epipolythiodioxopiperazine group of antibiotics.

Our interest in chetomin arose because we recovered isolates of *C. cochliodes* and *C. globosum*[9] from soil samples collected in Nova Scotia, where ruminant ill-thrift is a problem.[10] Laboratory cultures of the isolates were highly bacteriostatic[6] but bacteria resistant to chetomin[7] were also resistant to the antibiotics produced by the field isolates. Dr. Waksman gave us his original culture, and on cultivation of this fungus we obtained material closely similar to that reported by Geiger.[5] The physical and chemical properties of the antibiotic(s) produced by Dr. Waksman's strain of *C. cochliodes* were the same as those of the antibiotics produced by our field isolates. We have assumed the two materials to be identical and have adopted the name 'chetomin' for the antibiotics produced by the field isolates.

The amorphous ether-insoluble material reported by Geiger,[5] when subjected to t.l.c., showed two or more components when the chromatograms were sprayed with neutral aqueous silver nitrate. The relative amounts of these components varied in different fermentations, but usually the component of R_F 0·2 (5% HOAc–$CHCl_3$) predominated, and one of our field isolates produced this material exclusively. Poor recoveries of biological activity were obtained after column chromatography of crude chetomin on acid-washed alumina or silicic acid. However excellent recoveries were achieved when the crude material was partitioned on a Kieselguhr column in carbon disulphide–methanol–water (25 : 23 : 2),[11] and the weight distribution obtained is shown in Figure 1a. The sum of the weights of the fractions between the arrows in Figure 1a accounted for 73% of the material applied to the column. Reversed-phase partition chromatography of this material[11] gave the distribution shown in Figure 1b. The partition coefficient of the material eluted in the peak fractions of the distribution in the solvent benzene–chloroform–methanol–water (15 : 15 : 21 : 9) was 15·4; the partition coefficient calculated from the R_F value was 15·47. Rechromatography of the material eluted between 32 and 45 cm (Figure 1b) gave a weight distribution of identical R_F value, and a similar result was obtained when the tail fraction (55—65 cm) was rechromatographed. The specific optical rotation of fractions across the distribution lay in the range +319—326° and their $E^{1\%}_{1\,cm.}$ values in the range 130—135. All had the same biological activity, catalysed the decomposition of azide by iodine,[12] and gave *N*-methylalanine and sarcosine[11,13] on acid hydrolysis. We have been unable to obtain results with any combination of chromatographic solvent and adsorbents which suggest this material to be heterogeneous. Nevertheless the peak fractions from the distribution shown in Figure 1b did not give a diffraction pattern when irradiated with *X*-rays, and the i.r. spectrum of the material[14] confirmed its non-crystallinity. Elemental analysis suggested the formula $C_{31}H_{30}N_6O_6S_4,2H_2O$, and anhydrous material was obtained by precipitation from benzene with ether.

Chetomin readily gave a bistrimethylsilyl ether and an amorphous diacetate, the elemental analyses of which

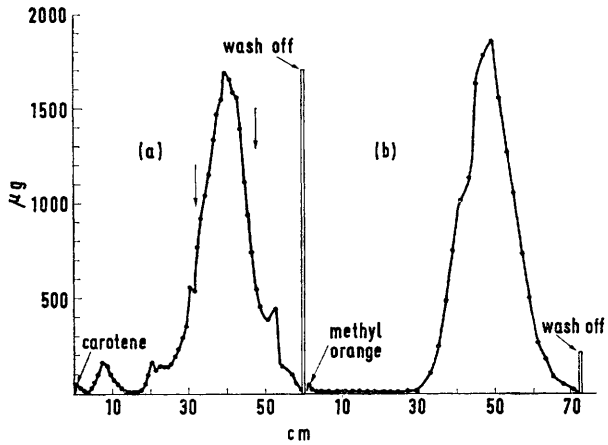


FIGURE 1 (a) Weight distribution of chetomin on a Hyflosupercel column (14·5 × 1 cm); solvent carbon disulphide–methanol–water (25 : 23 : 2); lower phase = mobile phase; (b) weight distribution of chetomin on a hydrophobic Hyflosupercel column (23 × 1 cm); solvent benzene–chloroform–methanol–water (15 : 15 : 21 : 9); upper phase = mobile phase

agreed with the foregoing empirical formula. The mass spectrum of the ester did not reveal a molecular ion; the ion of greatest mass had *m/e* 546 ($M - S_4 - 2HOAc$) which fragmented to abundant ions of *m/e* 281 and 265. The latter appeared as the base peak in the spectrum and was shown to lose 83 mass units in the next fragmentation step, an ion reaction typical of 2-methylenedioxopiperazines.[15] The diacetate was methylated with dimethyl sulphate to give an *N*-methyl derivative $(C_{18}H_{18}N_3O_4S_2)_n$; its n.m.r. spectrum indicated signals due to 36 protons, none of which were exchangeable with deuterium. Chetomin was easily reduced with sodium borohydride and the product obtained on methylation gave analytical figures corresponding to $C_{34}H_{40}N_6O_5S_4$.[16]

[6] D. Brewer, D. E. Hannah, and A. Taylor, *Canad. J. Microbiol.*, 1966, **12**, 1187.
[7] D. Brewer, D. E. Hannah, R. Rahman, and A. Taylor, *Canad. J. Microbiol.*, 1967, **13**, 1451.
[8] P. W. Trown, *Biochem. Biophys. Res. Comm.*, 1968, **33**, 402.
[9] D. Brewer, S. Safe, and A. Taylor, in preparation.
[10] D. Brewer, F. Calder, T. M. MacIntyre, and A. Taylor, *J. Agric. Sci.*, 1971, **76**, 465.
[11] R. L. M. Synge and E. P. White, *New Zealand J. Agric. Res.*, 1960, **3**, 907.
[12] D. Brewer and A. Taylor, *Canad. J. Microbiol.*, 1967, **13**, 1577.
[13] G. Lowe, A. Taylor, and L. C. Vining, *J. Chem. Soc.* (*C*), 1966, 1799.
[14] A. Taylor, 'Microbial Toxins,' vol. VII, Academic Press, New York, 1971, p. 349
[15] M. S. Ali, J. S. Shannon, and A. Taylor, *J. Chem. Soc.* (*C*), 1968, 2044.
[16] W. D. Jamieson, R. Rahman, and A. Taylor, *J. Chem. Soc.* (*C*), 1969, 1564.

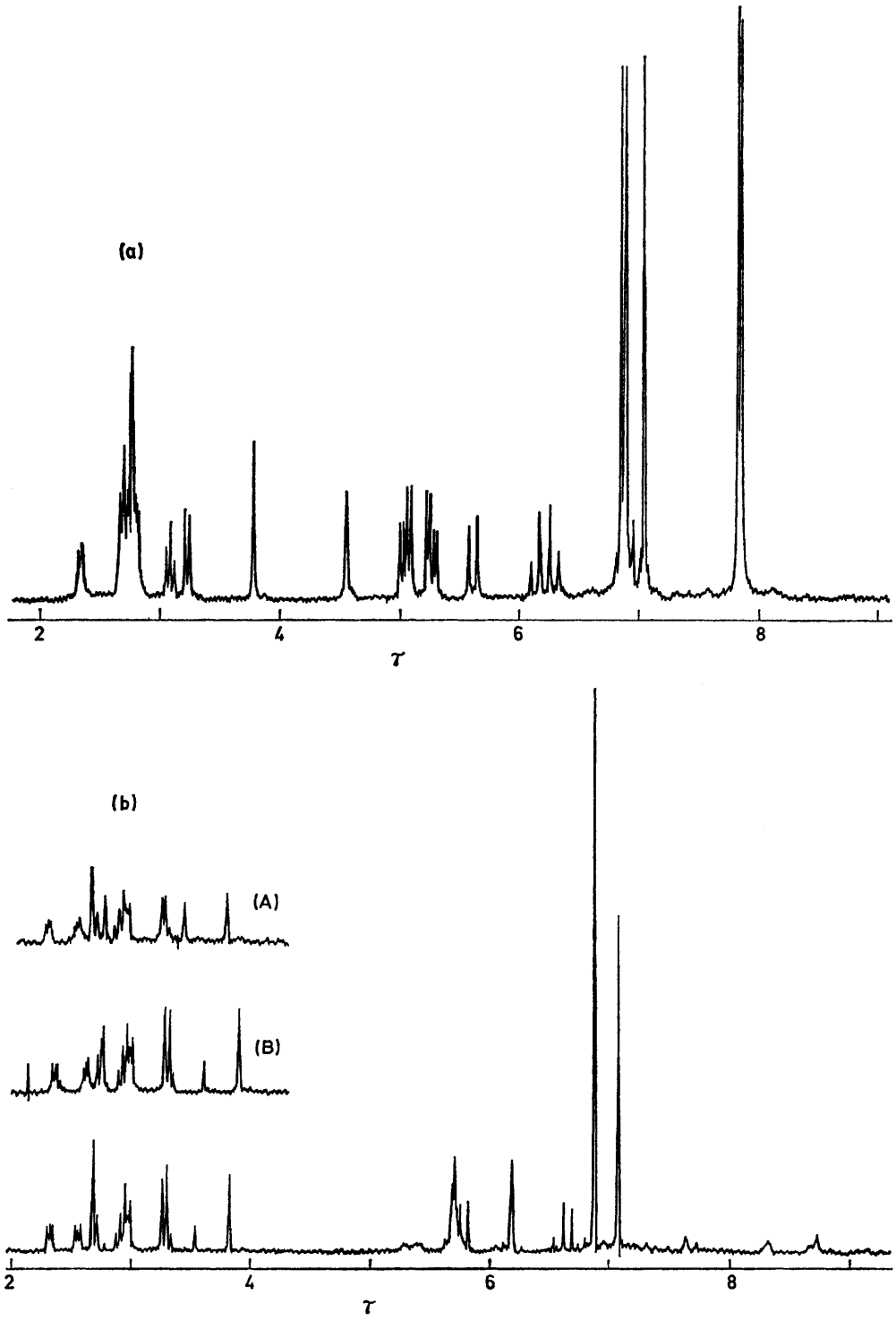


FIGURE 2 (a) N.m.r. spectrum of chetomin diacetate in $CDCl_3$; (b) n.m.r. spectra of chetomin in $(CD_3)_2CO$, (A) in the presence of 20% trispivalomethanatoeuropium(III); (B) in the presence of 20% trispivalomethanatopraseodymium(III)

The mass spectrum of this compound showed a molecular ion, *m/e* 740, in agreement with this formula. The spectrum also showed [16] the loss of four *S*-methyl groups, the presence of which was confirmed by n.m.r. spectroscopy. Acetylation of the tetra-*S*-methyl derivative gave a monoacetate.

The 220 MHz n.m.r. spectrum of chetomin diacetate in [^{2}H]chloroform is shown in Figure 2a. Signals due to five methyl groups are resolved; four AB quartets and two singlets at τ 4·66 and 3·90, the former showing exchange, are also readily identified. The spectrum of chetomin in [$^2H_6$]acetone (Figure 2b) shows signals for nine aromatic protons, based on the integral of the *N*-methyl signal at τ 7·03. Thus, in agreement with the analytical evidence, thirty protons are present in chetomin. The data suggest that chetomin is either a single molecular entity or a van der Waals complex of two or possibly three components of empirical formula $C_{31}H_{30}N_6O_6S_4$. Since van der Waals complexes are known to separate in the mass spectrometer [17] we incline to the former possibility and assume it in the following discussion.

Treatment of chetomin with triethyl phosphite gave a product elemental analysis of which suggested the formula $C_{31}H_{30}N_6O_6S_2$. The i.r. carbonyl absorptions of this compound were at 1720 cm^{-1}, whereas chetomin absorbed at 1685 cm^{-1}. Such a shift, due to the presence of a more strained ring system, has been shown to occur in the gliotoxin and sporidesmin series when an epidithiodioxopiperazine is converted into its epithio-derivative.[18]

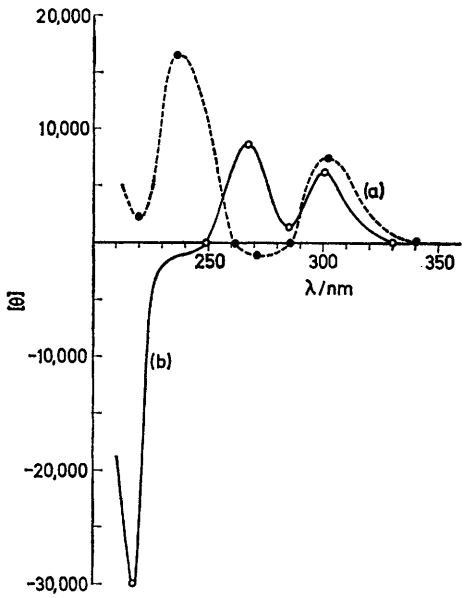
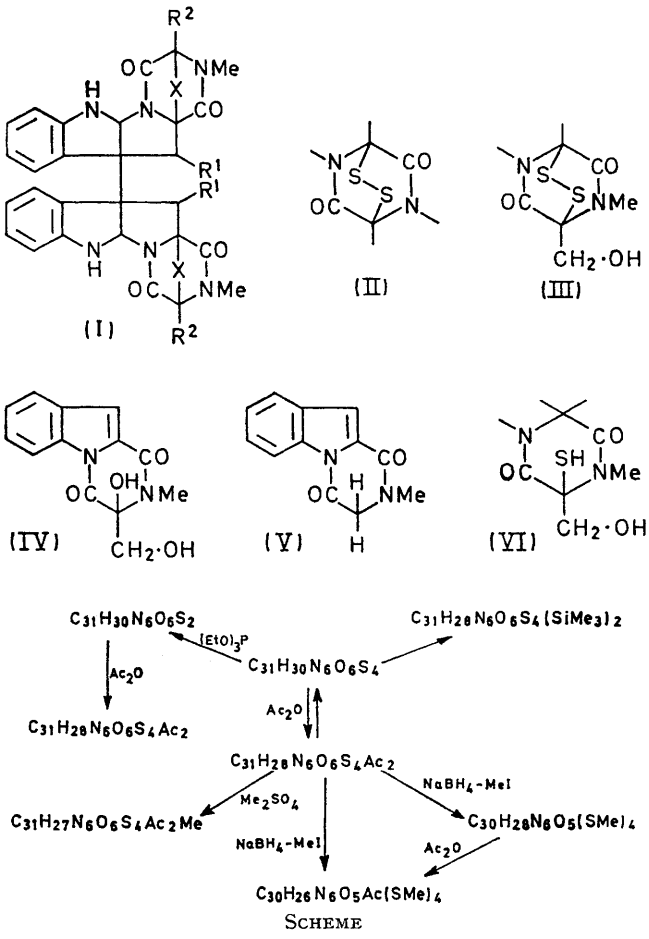


FIGURE 3 (a) C.d. of chetomin diacetate; (b) c.d. of dethiochetomin diacetate

The c.d. curve of chetomin (Figure 3) was almost the same as that reported for chaetocin [19] (I; $R^1 = H$, $R^2 = CH_2{\cdot}OH$, $X = S_2$) but the c.d. of the dethio-product (Figure 3) was in some respects enantiomorphic. Such isomerisation has been shown to occur in the reaction of triphenylphosphine and epidithiodioxopiperazines.[18] The dethiochetomin gave a diacetate. These reactions, summarised in the Scheme, indicate the presence of two epidithiodioxopiperazine rings [*i.e.* (II)] in chetomin.



SCHEME

Analysis showed the presence of three *N*-methyl groups in chetomin; this was confirmed by the n.m.r. spectrum (Figure 2b). Since the group (II) accounts for four of the six nitrogen atoms in the molecule, at least one of these carries a methyl substituent. A comparison of the n.m.r. spectrum of chetomin with that of chetomin diacetate (Figure 2) shows that a group of signals, equivalent to four protons is shifted downfield. The signals in the spectra of chetomin diacetate and deuteriated chetomin were much better resolved, and decoupling experiments showed the presence of two $CH_2{\cdot}OH$ groups (J_{AB} 12 Hz) of very similar chemical shift. The partial structure (III) occurs in dehydrogliotoxin, which on acid hydrolysis gives *N*-methylalanine.[13] This amino-acid was also obtained from chetomin, which similarly does not have a *C*-methyl

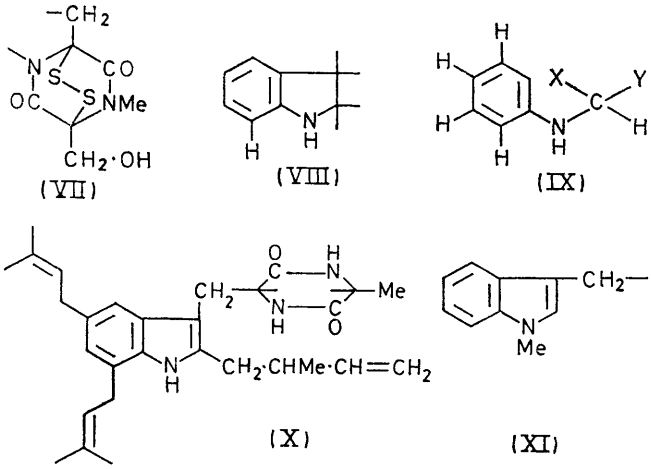
[17] O. Hutzinger and W. D. Jamieson, *Analyt. Biochem.*, 1970, **35**, 351.

[18] S. Safe and A. Taylor, *J. Chem. Soc.* (*C*), 1971, 1189.

[19] D. Hauser, H. P. Weber, and H. P. Sigg, *Helv. Chim. Acta*, 1970, **53**, 1061.

group (Figure 2), and it is therefore possible that one of the CH_2·OH groups in chetomin is part of a similar group, *i.e.* the fragment (III) is present in chetomin. This conclusion was supported by the formation of the tetra-*S*-methyl derivative $C_{34}H_{40}N_6O_5S_4$ where, in the course of the borohydride reduction, the group CH_2O was lost. It has been shown that treatment of the glycol (IV) with acid [15] results in a reverse aldol reaction to give the sarcosine derivative (V); an analogous reaction of the group (VI) might be expected. It seems reasonable, because of its similar chemical shift and coupling constant, to assign the other CH_2·OH group to an analogous position on the second dioxopiperazine ring. It would then follow, because only *N*-methylated amino-acids were obtained on hydrolysis, that another *N*-methyl group is located as in structure (III), and that two such groups are present in chetomin.

Double resonance experiments established the presence of two more methylene groups in chetomin. Their chemical shifts and coupling constants (J_{AB} 15 Hz) were similar to those of the pyrrolidine methylene groups in sporidesmin B (J 16 Hz) [20] and chaetocin (I; R^1 = H, R^2 = CH_2·OH, X = S_2).[19] The values of $\Delta\nu_{AB}$ for the two groups were different; in polar solvents the signals



for one of the groups appeared as a broad singlet (Figure 2b). It is suggested, tentatively, that both of these methylene groups are substituents on the dioxopiperazine rings and that one is in a stereochemically rigid conformation whilst the other is free to rotate. Thus the partial structure (III) may be expanded to (VII). It is also possible that the pendant methylene groups are bound to an atom that does not bear a hydrogen substituent since the protons of each methylene group are coupled only to one another.

The two groups (VII) account for $C_{14(16)}H_{16}N_4O_6S_4$, thus leaving the nature of the residual $C_{17(15)}H_{14}N_2$ to be found. When chetomin diacetate was treated with acetic anhydride a number of products were obtained which have not yet been adequately characterised. One gave a molecular ion corresponding to $C_{33}H_{28}N_6O_5$ and its n.m.r. spectrum indicated the presence of a *N*-acetyl group. In this spectrum the doublet (J 7·5 Hz)

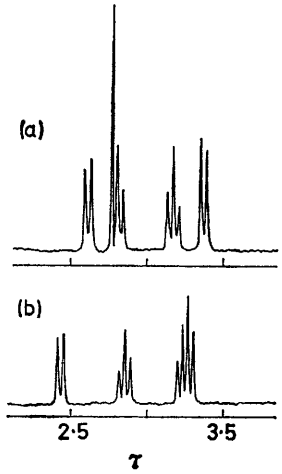


FIGURE 4 220 MHz N.m.r. spectra of aromatic protons of deuteriated chaetocin diacetate, (a) in $CDCl_3$; (b) in $(CD_3)_2CO$

due to one aromatic proton was shifted downfield from at least τ 2·32 to τ 1·79. Such behaviour is characteristic [21] of the group (VIII) on acetylation. Signals for two aromatic protons in the spectrum of chetomin diacetate are resolved in [^{2}H]chloroform (τ 3·20 and 3·31) (Figure 2a). The coupling constant of these protons (7·5 Hz) is typical of *ortho*-related aromatic protons, but the appearance of the signal for one as a triplet implies that it is also coupled to another *ortho*-proton. In the spectrum of chetomin in [$^2H_6$]acetone in the presence of trispivalomethanatopraseodymium(III) another doublet and triplet were seen, and this group of doublet, triplet, triplet, doublet, all with coupling constants of 7·5 Hz, is also found in the unusual first-order spectrum of the aromatic protons of chaetocin diacetate (Figure 4), where the downfield shift of the doublet at τ 3·31 in more polar solvents is also seen. The CH signal at τ 3·90 was significantly sharper when the NH group of chetomin diacetate was replaced by ND.

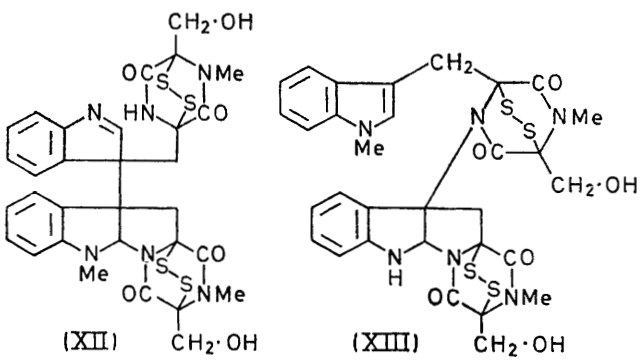
Similar changes in the n.m.r. spectra of chaetocin (I; R^1 = H, R^2 = CH_2·OH, X = S_2), its degradation product (I; R^1 = H, R^2 = CH_2·OH, X = H_2),[19] and verticillin A [22] (I; R^1 = OH, R^2 = Me, X = S_2) were observed when the NH systems in these molecules were replaced by ND. Thus the partial formula (IX), where the groups X and Y are not hydrogen or an atom bearing a hydrogen substituent, is probably present in chetomin. The group (IX), C_7H_6N, when subtracted from $C_{17(15)}$-$H_{14}N_2$, leaves the residue $C_{10(8)}N_8N$, which contains the remaining NMe group and the chromophore $\lambda_{max.}$ 295, 284, and 275 nm (no other group deduced so far would absorb in this frequency range). In the Table the fre-

[20] J. W. Ronaldson, A. Taylor, E. P. White, and R. J. Abraham, *J. Chem. Soc.*, 1963, 3172.
[21] K. Nagarajan, M. D. Nair, and P. M. Pillai, *Tetrahedron*, 1967, **23**, 1683.
[22] K. Katagiri, K. Sato, S. Hayakawa, T. Matsushima, and H. Minato, *J. Antibiotics*, 1970, **23**, 420.

	ε		
$\lambda_{max.}$/nm	Chetomin	Echinulin	Chaetocin diacetate
301			5400
295	8250	7700	
284	9600	9750	
275	9100	10,100	
240			14,200

quency and extinction coefficients of absorptions of chetomin and echinulin (X) [23] are compared. It is clear that the same chromophore is present in both, but absent in chaetocin. Thus the $C_{9(7)}H_5NMe$ residue is probably the chromophore (XI) where the methylene group is one of the pendant groups of (VII). The presence of the chromophore (XI) requires a singlet absorption for the proton at position 2 in the indole ring in the n.m.r. spectrum of chetomin and its derivatives. The n.m.r. spectra of the nine aromatic protons of chetomin in [$^2H_6$]acetone and in the presence of the shift reagents [24] trispivalomethanatoeuropium(III) (A) and trispivalomethanatopraseodymium(III) (B) are shown in Figure 2b. Only small shifts were observed in the case of eight of the nine protons, but the group of signals at about τ 2·7 consists of a doublet, referred to above (Figure 2b, A and B) and a singlet, which shifted downfield in the presence of the europium(III) reagent and upfield in the presence of the praseodymium(III) reagent, as expected. This evidence is regarded as support for the indolic fragment (XI) in chetomin.

The two epidithiopiperazine groups (VII), the indole fragment (XI), and the system (IX) account for $C_{30}H_{30}N_6O_6S_4$, thus a single quaternary carbon atom remains to be located. There are six valencies available in the



(XII) (XIII)

four groups (VII), (IX), and (XI); thus four are bound to this quaternary carbon atom and two to each other. This bonding involves (for valency reasons) two rings or double bonds, and all the possible arrangements can be easily drawn. Of these possibilities two [(XII) and (XIII)] are attractive biogenetically. Structure (XII) was suggested to us by Dr. Kis but it seems inadequate on the grounds that chetomin is not basic and its u.v. spectrum is the same in acid or neutral solution [25] (it is also unchanged in basic solution). We therefore currently favour structure (XIII).

[23] G. Casnati, A. Quilico, and A. Ricca, *Gazzetta*, 1962, **92**, 129.
[24] C. C. Hinckley, *J. Amer. Chem. Soc.*, 1969, **91**, 5160; J. K. M. Sanders and D. H. Williams, *Chem. Comm.*, 1970, 422.

EXPERIMENTAL

Spectroscopic measurements were made on the instruments and under the experimental conditions mentioned in Part XII unless otherwise stated. Thin (0·3 mm, and thick (0·8 mm) layer chromatography were carried out on silica gel (Merck) (20 × 100 cm plates). Samples of compounds from different sources were shown to be identical by comparison of their i.r., u.v., and n.m.r. spectra.

Chetomin.—(*a*) The production medium (99 l) consisted of (concentrations in g l^{-1}) sodium nitrate, 3; potassium chloride, 0·5; iron(II) sulphate heptahydrate, 0·01; sucrose, 30; dipotassium hydrogen phosphate, 1; magnesium sulphate heptahydrate, 0·5; calcium carbonate, 3; corn steep liquor (St. Lawrence Starch Co., Ontario), (5 ml), and water. The medium (1 l samples) was dispensed into 2 l Erlenmeyer flasks and inoculated with a chopped mycelial suspension (1 ml), four days old, of *Chaetomium cochliodes* HLX 833.[9] The cultures were incubated for 10 days on a rotatory shaker (220 rev. min^{-1}). The cultures were combined and filtered and the mycelium was suspended in water (10 l). Ice (0·5 kg) was added, the mixture was macerated in a Waring blender for 2 min, and the slurry was lyophilised. The dry material (1·69 kg) was macerated with benzene (4 l), the mixture was filtered, and the residue was similarly macerated twice more with benzene (2 × 4 l). The combined benzenoid extracts were evaporated at 30° and 10 mmHg and the residue (0·065 kg) was dissolved in a mixture of light petroleum (b.p. 40—60°; 1 l), methanol (950 ml) and water (50 ml). The mixture was filtered, the phases were separated, and the methanol phase was extracted with light petroleum (3 × 1 l). The methanol raffinate thus obtained was evaporated, the residue (14·8 g) was dissolved in chloroform (50 ml) and diethyl ether (150 ml) was added. The mixture was kept for 18 h at 4° and the precipitate {7·5 g; $E^{1\%}_{1\,cm.}$ 126; $[\alpha]_D^{22}$ +255° (*c* 0·5 in $CHCl_3$)} was collected. A portion (0·658 g) was dissolved in carbon disulphide–methanol–water (25 : 23 : 2; 45 ml) and carotene (10 mg) was added, followed by purified Hyflosupercel [11] (10 g). The mixture was packed on the top of a column (4·2 cm diam., 100 g Hyflosupercel, retention volume 190 ml), prepared as described by Ronaldson *et al.*[20] The temperature of the column was kept at 13° ± 0·1° by means of a water jacket. Fractions were collected, after the appearance of carotene in the eluate, as the solvent above the column descended each 10 cm. Fractions 1—6 were discarded and the next seven fractions were collected, combined and evaporated (yield 0·48 g, 73% of the sample applied to the column). A portion (0·93 g; $E^{1\%}_{1\,cm.}$ 121, $[\alpha]_D^{22}$ +309°) was dissolved in the bottom phase of the system benzene–chloroform–methanol–water (15 : 15 : 21 : 9; 1 ml), and sufficient hydrophobic Hyflosupercel [26] was added to make a paste. A solution (0·1 ml) of Methyl Orange (15 mg) in the lower phase was added and the resulting mixture was packed on the top of a column of hydrophobic Hyflosupercel (28 g; 32 ml bottom phase, 2 × 40 cm, retention volume 33 ml). The temperature of the jacketed column was maintained as before at 14°, and an air pressure of 5 lb in^{-2} was applied to the top. The column was eluted with the top phase of the solvent system and fractions were measured by the distance of descent of the solvent above the column. Six 6 cm fractions were discarded after the

[25] B. Witkop and J. B. Patrick, *J. Amer. Chem. Soc.*, 1951, **73**, 1559.
[26] G. A. Howard and A. J. P. Martin, *Biochem. J.*, 1950, **46**, 535.

appearance of the Methyl Orange in the eluate; the next six fractions were combined, and evaporated. The residue (65 mg, 70% recovery; $E_{1\,cm.}^{1\%}$ 130, $[\alpha]_D^{22}$ +326°) was dissolved in chloroform (1 ml) and diethyl ether (3 ml) was added dropwise. The mixture was kept at 4° for 24 h and the colourless solid (28 mg) was collected (Found: C, 50·0, 50·3; H, 4·5, 4·4; N, 11·1, 11·3; S, 16·6; NMe, 5·8, 6·1. $C_{31}H_{30}N_6O_6S_4,2H_2O$ requires C, 49·9; H, 4·6; N, 11·3; S, 17·2; NMe, 6·0%), $E_{1\,cm.}^{1\%}$ 133, $[\alpha]_D^{22}$ +321°. When the fractions from the reversed-phase partition column were taken up in benzene (1 ml) and precipitated with diethyl ether, *chetomin* separated as an amorphous glass-like solid (Found: C, 52·0; 52·1, 52·2; H, 4·6, 4·7; N, 11·4, 11·5; O, 13·7, 13·8, 14·0; S, 17·9, 17·8. $C_{31}H_{30}N_6O_6S_4$ requires C, 52·4; H, 4·2; N, 11·8; O, 13·5; S, 18·2%); i.r. spectrum in ref. 14, u.v. in Table 1, c.d. in Figure 3, n.m.r. in Figure 2a.

(*b*) Chetomin diacetate (see later; 0·1 g), benzene (10 ml), and dimethyl sulphate (1 ml) were stirred together at 20° for 24 h. The solution was poured on to a silicic acid column, which was developed with benzene (500 ml) and then eluted with ethyl acetate (500 ml). The eluate was evaporated and the residue purified by t.l.c. (ethyl acetate–benzene, 1 : 3). The main band was eluted and the solution evaporated; the residue (40 mg) in benzene gave chetomin on addition of ether.

Chetomin Diacetate.—Chetomin (0·5 g), pyridine (3 ml), and acetic anhydride (1 ml) were mixed and kept for 12 h at 20°. Methanol (2 ml) was added, the solution was poured into water, and the mixture was extracted with chloroform. The extract was washed with dilute hydrochloric acid, dried (Na_2SO_4), and evaporated; the residue was dissolved in benzene and the *diacetate* (0·52 g), m.p. 175—177°, was precipitated with ether (Found: C, 52·5; 52·4; H, 4·4, 4·2; N, 10·0, 10·0; O, 16·1, 16·0; S, 15·6, 15·5. $C_{35}H_{34}N_6O_8S_4$ requires C, 53·0; H, 4·3; N, 10·5; O, 16·1; S, 16·1%), $\lambda_{max.}$ (MeOH or 0·1N-HCl in MeOH) 274, 285, and 293 nm (log ε 3·95, 4·00, and 4·16), $\nu_{max.}$ (KBr) 1755, 1695, 1610, 1225, and 1040 cm^{-1}; n.m.r. spectrum in Figure 2a.

N-*Methylchetomin Diacetate.*—Chetomin diacetate (0·2 g) was dissolved in dimethyl sulphate (2 ml) and heated for 5 min at 95°. The mixture was immediately diluted with benzene (10 ml) and poured on a silicic acid column (50 g). The column was washed with benzene (500 ml) and eluted with ethyl acetate (800 ml). The eluate was evaporated and the residue purified by t.l.c. (benzene–ethyl acetate, 4 : 1; developed three times). Two bands were obtained, the most polar (90 mg) was chetomin diacetate and the less polar was rechromatographed (t.l.c.; $CHCl_3$–Bu^tOH, 99 : 1; developed three times). Material (40 mg) obtained by elution of the non-polar band was dissolved in benzene, and N-*methylchetomin diacetate* was precipitated with ether, m.p. 112—114° (Found: C, 54·0; H, 4·8; N, 10·3; S, 15·5. $C_{36}H_{36}N_6O_8S_4$ requires C, 53·5; H, 4·5; N, 10·4; S, 15·8%), $\lambda_{max.}$ (MeOH) 272, 280, and 289 nm (log ε 4·15, 4·15, and 4·07), $\nu_{max.}$ (KBr) 1750, 1680, 1605, 1220, and 1035 cm^{-1}, τ ($CDCl_3$) 2·2—3·6 (10, m), 4·91, 5·11, 5·20, and 5·40 (4H, *J* 12 Hz), 5·84, 6·10, 6·19, and 6·43 (4H, *J* 15 Hz), 6·87 (6H), 7·00 (6H), and 7·90 (6H).

Chetomin Bistrimethylsilyl Ether.—Chetomin (0·2 g), pyridine (3 ml), and trimethylsilyl chloride (1 ml) were mixed and kept for 12 h at 20°. The mixture was poured into water and the products were extracted with chloroform. The extract was washed with dilute hydrochloric acid, dried (Na_2SO_4), and evaporated, and the residue was recrystallised from methanol to give plates of the *bistrimethylsilyl ether* (0·18 g), m.p. 153—155° (Found: C, 51·8; H, 5·25; N, 9·4; S, 15·4. $C_{37}H_{46}N_6O_6S_4Si_2$ requires C, 52·0; H, 5·4; N, 9·4; S, 15·0%), $\lambda_{max.}$ (MeOH) 274, 285, and 293 nm (log ε 4·11, 4·13, and 4·11), $\nu_{max.}$ (KBr) 1700, 1615, and 1260 cm^{-1}, τ [$(CD_3)_2CO$] 2·0—3·4 (9H, m) 3·52 (1H), 3·73 (1H, d, *J* 1 Hz), 5·52 (4H, d, *J* 1·5 Hz), 6·10 (2H), 5·80, 6·10, 6·53, and 6·83 (4H, *J* 21 Hz), 6·80 (6H), 7·03 (3H), and 9·77 (18H).

Reaction of Chetomin with Triethyl Phosphite.—Chetomin (0·3 g) was dissolved in tetrahydrofuran (1 ml) and triethyl phosphite (1 ml) was added. After 1 h at 22° light petroleum (30 ml) was added; the product was collected (0·21 g) and chromatographed (t.l.c.; three plates; benzene–ethyl acetate, 1 : 1). The main band was extracted; material obtained was dissolved in tetrahydrofuran and *dethiochetomin* was precipitated by addition of ether; m.p. 213—215° (Found: C, 57·0; H, 4·8; N, 12·9; S, 10·2. $C_{31}H_{30}N_6O_6S_2$ requires, C, 57·5; H, 4·7; N, 13·0; S, 9·9%), $\lambda_{max.}$ (EtOH) 274, 285, and 294 nm (log ε 3·90, 3·92, and 3·90), $\nu_{max.}$ (KBr) 3425, 1720, and 1610 cm^{-1}; c.d. data in Figure 3; τ [$(CD_3)_2CO$] 2·0—3·4 (9H, m), 3·53 (1H), 4·20 (1H, d, *J* 1·5 Hz), 5·83 (4H), 5·88 and 6·85 (2H, J_{AB} 12 Hz), 6·4 (2H), 6·91 (3H), 7·14 (3H), and 7·16 (3H).

Dethiochetomin Diacetate.—Dethiochetomin (0·2 g), pyridine (1 ml), and acetic anhydride (0·3 ml) were mixed, kept for 12 h at 22°, and then diluted with methanol (1 ml). The mixture was poured into water and the products were extracted with chloroform. The crude product was chromatographed (t.l.c.; two plates; ethyl acetate–benzene, 1 : 2) and the *acetate* was precipitated from benzene with ether, m.p. 141—143° (Found: C, 57·6; H, 4·6; N, 11·1; S, 8·8. $C_{35}H_{34}N_6O_8S_2$ requires C, 57·5; H, 4·65; N, 11·5; S, 8·8%), $\lambda_{max.}$ 273, 285, and 294 nm (log ε 3·94, 3·96, and 3·94), $[\theta]_{300}$ +8000°, $[\theta]_{270}$ +8000°, $[\theta]_{242}$ −5100°, $[\theta]_{215}$ −31,000°, (*c* 0·0099 in dioxan), $\nu_{max.}$ (KBr) 1755, 1725, 1610, 1225, and 1050 cm^{-1}, τ ($CDCl_3$) 2·1—3·3 (9H, m), 3·55 (1H), 4·16 (d, *J* 1 Hz), 5·25 (4H), 5·80 and 6·80 (2H, d, *J* 16 Hz), 6·37 (2H), 6·90 (3H), 7·10 (3H), 7·16 (3H), 7·92 (3H), and 7·98 (3H).

Deformyltetra-S-*methylchetomin.*—Chetomin (0·2 g), pyridine (1 ml), methanol (4 ml), and methyl iodide (1 ml) were stirred together under nitrogen, and sodium borohydride (0·05 g) was added during 30 min. Methyl iodide (1 ml) was added and the solution was stirred for 3 h and poured into water. The products were extracted with chloroform. The extract was washed with dilute hydrochloric acid, dried (Na_2SO_4), and evaporated, and the residue was chromatographed (t.l.c.; benzene–ethyl acetate 4 : 1). The product (0·19 g) was eluted and dissolved in benzene, and *deformyltetra*-S-*methylchetomin* was precipitated with ether; m.p. 179—181° (Found: C, 54·8; H, 5·1; N, 10·75; S, 17·2. $C_{34}H_{40}N_6O_5S_4$ requires C, 55·1; H, 5·4; N, 11·35; S, 17·3%), *m/e* 740, 695, 694, 693, 648, 647, 646, 603, 602, 601, 600, 599, 555, 554, 553, 552, and 465, $\lambda_{max.}$ (MeOH) 283, 287, and 294 nm (log ε 3·79, 3·83, and 3·84), τ ($CDCl_3$) 2·3—3·4 (9H, m), 3·90 (1H, d, *J* 1 Hz), 4·71 (1H, d, *J* 1 Hz), 5·6—6·9 (7H, m), 6·81 (3H), 6·88 (3H), 7·20 (3H), 7·70 (3H), 7·78 (3H), 7·90 (3H), and 8·00 (3H).

Deformyltetra-S-*methylchetomin Acetate.*—(*a*) Chetomin diacetate (0·25 g), pyridine (1 ml), methanol (3 ml), and methyl iodide (2 ml) were stirred together under nitrogen and sodium borohydride was added during 30 min. Methyl iodide (1 ml) was then added and the mixture was stirred for 3 h. It was poured into water and the products were extracted with chloroform. The extract was washed with dilute hydrochloric acid, dried (Na_2SO_4), and evaporated,

and the residue was purified by t.l.c. (benzene–ethyl acetate, 4 : 1). The main band was eluted; the product was dissolved in benzene and *deformyltetra*-S-*methylchetomin acetate*, m.p. 130—132°, was precipitated with ether (Found: C, 55·7; H, 5·4. $C_{36}H_{42}N_6O_6S_4$ requires C, 55·2; H, 5·4%), $\lambda_{max.}$ (MeOH) 274, 283, and 287 nm (log ε 3·80, 3·83, and 3·84), $\nu_{max.}$ (KBr) 1750, 1660, 1605, 1230, and 1065 cm^{-1}, τ ($CDCl_3$) 2·2—3·5 (9H, m), 3·72 (1H, d, *J* 1 Hz), 4·80 (1H), 5·12, 5·43 (2H, *J* 14 Hz), 5·78 and 6·73 (2H, *J* 15 Hz), 6·20 (2H), 6·42 (1H), 6·78 (3H), 6·91 (3H), 7·10 (3H), 7·68 (3H), 7·72 (3H), 7·83 (6H), and 8·02 (3H).

(*b*) Deformyltetra-*S*-methylchetomin (0·2 g), pyridine (3 ml) and acetic anhydride (1 ml) were mixed and kept for 12 h at 20°. The solution was poured into water and the product was extracted with chloroform; the extract was washed with dilute hydrochloric acid, dried (Na_2SO_4), and evaporated. Deformyltetra-*S*-methylchetomin acetate, m.p. 130—132° was obtained after chromatography of the residue (t.l.c.; benzene–ethyl acetate, 4 : 1).

Anhydrodethiochetomin.—Chetomin acetate (0·8 g), acetic acid (6 ml), and acetic anhydride (2 ml) were heated together at 110° for 16 h. The mixture was cooled, diluted with methanol (2 ml), and evaporated. Chromatography of the residue (t.l.c.; benzene–diethyl ether, 3 : 2; developed three times) gave three bands. The least polar (0·09 g) was sulphur; the most polar yielded material which was dissolved in benzene. Anhydrodethiochetomin was precipitated with ether; m.p. 200—202°, *m/e* 588·216 ($C_{33}H_{28}N_6O_5$ requires 588·212), τ [$(CD_3)_2CO$] 1·73 and 1·86 (1H, *J* 7·5 Hz), 2·20—2·94 (7H, m), 2·96 (1H), 3·12 (1H), 4·24, 4·26, 4·86, and 4·88 (2H, *J* 1·5 Hz), 4·41, 4·42, 5·02, and 5·03 (2H, *J* 1 Hz), 6·76 (6H), 7·03 (3H), and 7·51 (3H).

Chaetocin Diacetate.—Chaetocin [19] (0·2 g), pyridine (3 ml), and acetic anhydride (1 ml) were mixed and kept for 12 h at 20°. The mixture was worked up as described for chetomin diacetate and gave chaetocin diacetate, m.p. 227—229° (Found: C, 52·1; H, 4·2; N, 10·5; S, 16·6. Calc. for $C_{34}H_{30}N_6O_8S_4$: C, 52·6; H, 3·85; N, 10·8; S, 16·5%); n.m.r. spectrum ($CDCl_3$) as in Figure 4, and τ 5·12 and 5·43 (4H, *J* 12·5 Hz), 6·14 and 7·28 (4H, *J* 15 Hz), 6·98 (6H), and 7·90 (6H), $\nu_{max.}$ 1750, 1700, 1610, and 1225 cm^{-1}.

N-*Methylalanine*.—Chetomin (0·1 g) was dissolved in acetic acid (1 ml) and concentrated hydrochloric acid (2 ml) was added. The solution was heated at 110° for 36 h and was then evaporated. The residue was digested with water (2 ml) and the solution was run on to a cation exchange resin (Amberlite IR 120). The column (1 × 20 cm) was washed with water (200 ml) and then with dilute acetic acid (100 ml). The eluate was evaporated and a solution of the residue in water (2 ml) was run on to an anion exchange resin (Amberlite 400) column (1 × 20 cm). The column was washed with water (200 ml) and then with N-ammonium hydroxide (100 ml). The ammoniacal eluate was evaporated, the amino-acid residue was dissolved in water, and *N*-methylalanine (m.p. 279°) was separated by paper chromatography as previously described.[13]

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