Structure Determination of Two Extractives from *Aspergillus amstelodami* by Nuclear Magnetic Resonance Spectroscopy

By GIUSEPPE GATTI*

(*Istituto di Chimica delle Macromolecole del C.N.R., Via Alfonso Corti*, 12, 20133 *Milano, Italy*)

and ROSANNA CARDILLO, CLAUDIO FUGANTI, and DARIO GHIRINGHELLI

(*Istituto di Chimica del Politecnico, Centro del C.N.R. per la Chimica delle Sostanze Organiche Naturali*, 20133 *Milano, Italy*)

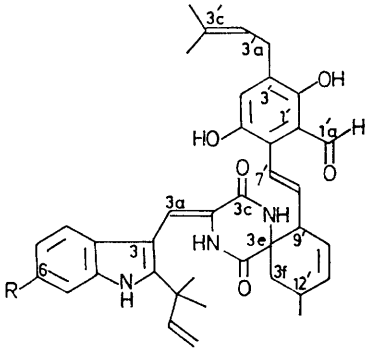
Summary Spectral evidence supports the assignment of structures (**1**) and (**2**) to two substances extracted from the mycelium of *Aspergillus amstelodami*; they can be obtained by thermal condensation of auroglaucine with neoechinuline B and neoechinuline C, respectively, which are all known metabolites of the fungus.

AMONG the isoprenylated dehydrotryptophan derivatives isolated from the mycelium of *Aspergillus amstelodami* two optically inactive compounds, referred to previously[1] as cryptoechinuline B and D, seemed interesting owing to their apparent molecular complexity. We report now spectral evidence which allows the assignment of structures (**2**) and (**1**), respectively to these compounds.

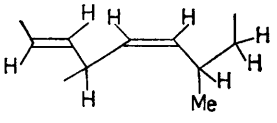
(i) The ^{1}H n.m.r. spectrum of (**1**) [270 MHz, 0·05 M, $(CD_3)_2SO$] shows the presence of 41 hydrogen atoms, in agreement with the $C_{38}H_{41}N_3O_5$ molecular composition determined by exact mass measurements. Five protons (δ 11·8, 10·9, 9·2, 8·8, and 7·8) are exchanged upon addition of 5% D_2O.

(ii) The ^{1}H signals at δ 8·8 and 7·8 suggest the presence of two CONH groups, in agreement with the two CO absorp-

tions in the ^{13}C n.m.r. spectrum [22·6 MHz, 0·3 M, $(CD_3)_2SO$] at δ 161·5 and 167·8 p.p.m. Moreover, the ^1H signal at δ 10·9 is in the range expected for tryptophan NH, and the aromatic ^1H signals between δ 6·9 and 7·5 form a 4-spin pattern typical of the benzene ring of tryptophan. The system of 3 protons at δ 1·4 and a ^{13}C singlet at δ 38·6 p.p.m. suggest the presence of the αα-dimethylallyl chain in position 2 of tryptophan, as in all compounds of the echinuline series. Finally, the ^1H absorption at δ 6·97 (3a-H) and the two carbon absorptions at δ 144·4 p.p.m. (C-3b) and 111·7 p.p.m. (C-3a), together with all the above data, show the existence of the dehydrotryptophanyl residue linked with another amino-acid residue to form a diketopiperazine ring, as in the neoechinuline series.[2]



(1) R = H
(2) R = 



(3)

(iii) For the remaining proton signals, analysis of the pattern assigned to fragment (**3**) was obtained by double resonance experiments (decoupling and INDOR, starting from 7'-H, which absorbs at δ 6·7) and by considerations of chemical shifts and coupling constants.

(iv) The presence of a γγ-dimethylallyl chain can be deduced from the proton pattern for $-CH_2CH=$ (δ 3·2 and 5·2) and from the methyl resonance which is diagnostic in both the ^{13}C (δ 17·7 and 25·6 p.p.m.) and the ^1H spectrum (δ 1·6).

(v) A ^{13}C signal at δ 197·3 p.p.m. with a doublet structure owing to coupling with the signal at δ 10·2 indicates the presence of a CHO group.

(vi) A ^{13}C singlet at δ 59·2 p.p.m. shows the presence of a quaternary carbon bonded to a substituent with a deshielding effect.

(vii) Comparison of the total number of carbon atoms (32) in the above-mentioned fragments with that given by the molecular formula and by the whole ^{13}C spectrum (38) shows that six more unsaturated carbon atoms are present in (**1**); this means that the number of unsaturations is 15, and consequently the number of rings present is 5, as given by the formula $(2C + N - H + 2)/2 - 15$. Since there are two rings in the tryptophan unit and one in the diketopiperazine unit, the remaining part of the molecule must contain two rings. This conclusion can be rationalized by postulating the presence of a penta-substituted benzene ring linked to fragment (**3**), fragment (**3**) forming a cyclohexene ring containing the quaternary carbon atom with the ^{13}C signal at 59·2 p.p.m. The other four substituents on the aromatic ring are the γγ-dimethylallyl chain mentioned already, the formyl group, and the phenolic OH groups. The presence of the latter groups is shown by the shifts of the exchangeable protons at δ 9·2 and 11·8 and by the shifts of the *ipso* carbon atoms at δ 147·1 and 153·1 p.p.m., while the only hydrogen atom attached directly to the ring appears in the ^1H spectrum as a singlet at δ 7·02. However, the substitution pattern in the aromatic ring cannot be determined by the above results.

(viii) The ^1H spectrum of (**2**) ($C_{43}H_{49}N_3O_5$, by exact mass measurements) is similar to that of (**1**), with the addition of two Me resonances at δ 1·7 and a $-CH_2CH=$ pattern at δ 3·3 and 5·3. Furthermore, the aromatic absorption of the tryptophan unit is reduced to an ABX system similar to that in neoechinuline C.[2] All these facts point to the presence of a γγ-dimethylallyl chain in position 6.

(ix) Consideration of the type of molecular framework and substitution patterns assigned from the above reasonings to the two substances suggested that these optically inactive extractives could be derived from auroglaucine[4]† and neoechinuline B[3] and C[2b] by regiospecific Diels–Alder condensation of the terminal diene of the side chain of the former on to the dehydroalanine units of the latter compounds. Indeed, when auroglaucine and neoechinuline B and C, respectively, were heated in an evacuated sealed tube at 150 °C overnight, a crude mixture was obtained from which condensation products identical (mixed m.p., chromatographic behaviour, mass spectra) to (**1**) and (**2**) were separated after SiO_2 chromatography. This evidence allows the assignment of the aromatic substitution pattern in the two extractives.

The compounds seem to arise from natural dehydroamino-acid derivatives at some stage in the extraction of the fungal mat, thus representing a new feature of the latter increasingly important class of natural products.[5]

We thank Professor Salvatori, E.N.I., Milano, for the exact mass measurements.

(*Received, 12th March* 1976; *Com.* 256.)

† In formulae (**1**) and (**2**) the framework derived from auroglaucine is primed.

[1] R. Cardillo, C. Fuganti, D. Ghiringhelli, and P. Grasselli, *Chimica e Industria*, 1975, **57**, 687.

[2] (a) M. Barbetta, C. Casnati, A. Pochini, and A. Selva, *Tetrahedron Letters*, 1969, 4457; (b) R. Cardillo, C. Fuganti, G. Gatti, D. Ghiringhelli, and P. Grasselli, *Tetrahedron Letters*, 1974, **3163**; (c) A. Dossena, R. Marchelli, and A. Pochini, *J.C.S. Chem. Comm.*, 1974, 771; (d) R. Cardillo, C. Fuganti, D. Ghiringhelli, P. Grasselli, and G. Gatti, *ibid.*, 1975, 778; (e) A. Dossena, R. Marchelli, and A. Pochini, *Experientia*, 1975, **31**, 1294.

[3] H. Itokawa, Y. Akita, and M. Yamazaki, *Yagugaku Zasshi*, 1973, **93**, 1251.

[4] H. Raistrick, R. Robinson, and A. R. Todd, *J. Chem. Soc.*, 1937, 80; A. Quilico, C. Cardani, and L. Panizzi, *Atti accad. Naz. Lincei Rend. Classe sci. fis, mat. e nat.*, 1953, **14**, 358.

[5] B. W. Bycroft and G. R. Lee, *J.C.S. Chem. Comm.*, 1975, 121, and ref. 5 therein.