Tetrahedron Letters,Vol.23,No.20,pp 2095-2098,1982 0040-4039/82/202095-04$03.00/0
Printed in Great Britain

ISOLATION OF ASPIROCHLORINE (=ANTIBIOTIC A30641) POSSESSING A NOVEL DITHIODIKETOPIPERAZINE STRUCTURE FROM ASPERGILLUS FLAVUS

Kanzo Sakata,* Hajime Masago,# Akira Sakurai and Nobutaka Takahashi
The Institute of Physical and Chemical Research, Wako-shi, Japan.
Faculty of Agriculture, Kyoto Prefectural University,
Sakyo-ku, Kyoto 606, Japan.

Summary: A very unusual dithiodiketopiperazine structure (1) has been assigned to aspiroclorine, $C_9H_{12}N_2O_5S_2Cl$, produced as a biologically active substance together with canadensolide by Aspergillus flavus, which has been found to be identical with the antibiotic A 30641 from A. tamarii.

Two bilogically active principles[1] against phytopathogenic fungi, Phytophthora spp. have been isolated from the culture filtrate of Aspergillus flavus.[2] The less polar compound, $C_{11}H_{14}O_4$, $[\alpha]_D^{21}$ -182°, has been identified as canadensolide which was isolated from culture broth of fungi, Penicillium canadense[3] and A. tamarii NRRL 8101.[4] The polar active principle named aspirochlorine (1): $[\alpha]_D^{21}$ +66.7° (c=0.33, MeOH); λ_{max}^{MeOH} nm (ε) 250 (sh., 4800), 298 (6300), 305 (sh., 5600); λ_{max}^{+NaOMe} nm (ε) 263 (sh., 7600), 313 (8000); ν_{max} ($CHCl_3$) 1726, 1622, 1604 cm^{-1}; m/z 360 (M^+, $C_{12}H_9N_2O_5S_2Cl$),[5] 296 (M-S_2, $C_{12}H_9N_2O_5S_2Cl$), 265, 241, 221, 210, 209, 182, 181; δ($CDCl_3$) 3.96 (3H, s), 4.89 (1H, d, 1.2), 5.15 (1H, d, 4.6), 5.97 (1H, br.), 6.78 (1H, s), 7.14 (1H, d, 1.2), 7.30 (1H, br.); $[\theta]_{231}^{21}$ +17,000, $[\theta]_{249}$ -23,000, $[\theta]_{269}$ +2900, $[\theta]_{280.5}$ ±0, $[\theta]_{303}$ +11,000, was concluded by comparison of their spectroscopic data to be identical with the antibiotic A 30641 isolated from A. tamarii NRRL 8101,[4,6] to which the structure 2 had been assigned.[6]

However, the ^{13}C NMR data of 1 we obtained could not be explained by the structure 2. All the signals (Fig.) were reasonably assigned except for a signal (δ 102.6) which must be ascribed to the tertiary bridgehead carbon (C-8).[7] ^{13}C NMR spectra of a few epidithiodiketopiperazine compounds have been reported[8] and the signal of the particular carbon is observed in a region δ 74-78. The

* Present address: Department of Agricultural Chemistry, Faculty of Agriculture, Shizuoka University, 836 Ohya, Shizuoka 422, Japan.

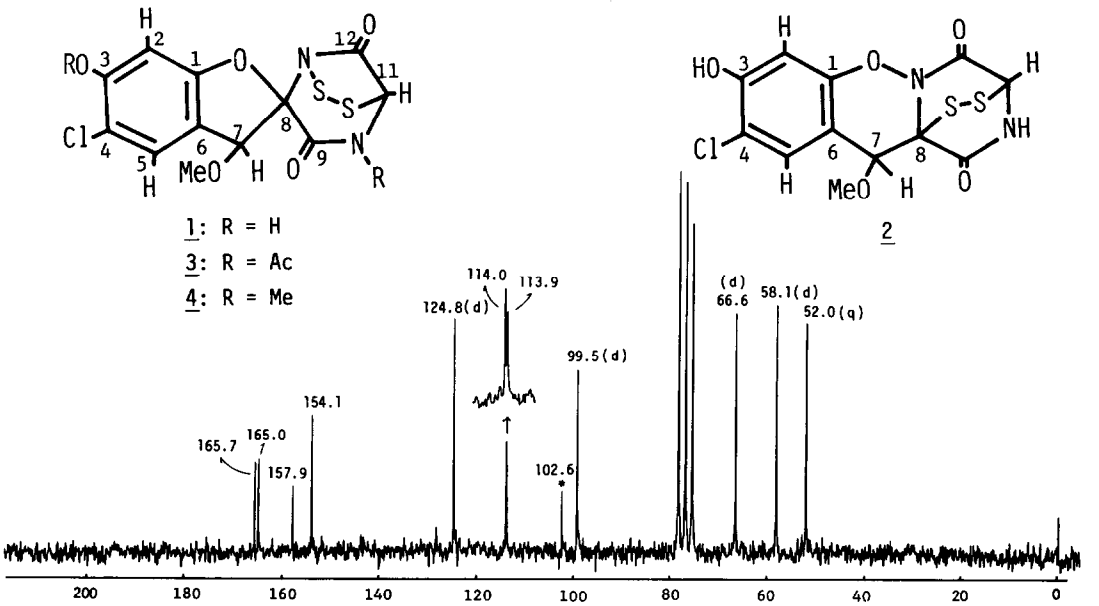


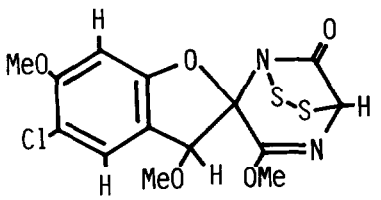
Fig. C-13 NMR Spectrum of 1 (22.53 MHz, $CDCl_3$, PND).

difference of the chemical shift is too big to be rationalized by the effect of the N-O linkage. This fact together with the extremely high carbonyl absorption (1725 cm^{-1}) for the amide carbonyl[9] has led us to conclude that the structure 2 proposed for the active principle must be revised.

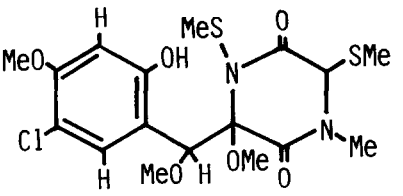
Coloration with a $AgNO_3$ reagent[10] together with the ions of m/z 256 ($M-S_2$) and 64 (S_2)[11] in the mass spectrum of 1 indicated the presence of a disulfide linkage. The bathochromic shift (15 nm) in alkaline conditions showed a phenolic hydroxyl group in 1.

Acetylation with acetic anhydride and pyridine gave an N,O-diacetyl derivative (3): m/z 444 (M^+, $C_{16}H_{13}N_2O_7S_2Cl$), 380 ($M-S_2$); $\delta(CDCl_3)$ 2.37, 2.72 (3H each s). Amino acid analysis of the acid hydrolysis product of desulfurized 1 gave glycine and an unidentified aromatic amino acid, which suggests the presence of a diketopiperazine structure in 1.

In the ^1H NMR spectrum of 1 were observed a methoxyl signal (δ 3.96) and two olefinic proton signals (δ 6.78, s and 7.14, d, 1.2), one of which is coupled to a methine proton (δ 4.89, d, 1.2). Another methine proton signal (δ 5.15, d) shifted downfield to δ 6.21 changing into singlet on acetylation. The tertiary carbon signal (δ 102.6) should be assigned to a carbon with a partial structure $>C<^{O-}_{X-}$ (X=O or N). Considering biosynthesis of a diketopiperazine compound and all the spectroscopic data described above allow us to propose a quite unusual structure 1 for aspirochlorine. The following reactions on 1 support the structure.

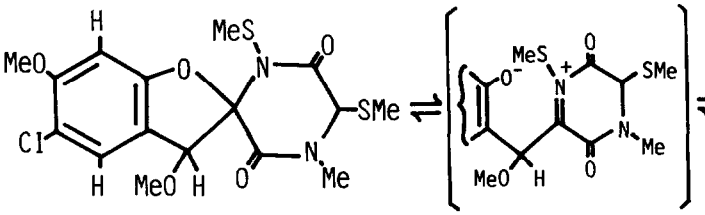


5

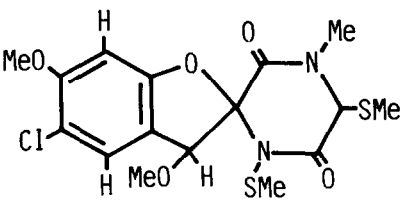


8

NaOMe/MeOH



6 or 7



6 or 7

Treatment of 1 with ethereal diazomethane in MeOH yielded an N,O-dimethyl (4) and an O,O-dimethyl (5) derivatives. 4: main product; m/z 388 (M^+, $C_{14}H_{13}N_2O_5S_2Cl$), 324 (M-S_2, $C_{14}H_{13}N_2O_5Cl$); δ ($CDCl_3$) 3.12, 3.92, 3.97 (3H each, s), 4.92 (1H, d, 1.2), 5.00, 6.72 (1H each, s), 7.16 (1H, d, 1.2); λ_{max}^{MeOH} nm 259 (sh.), 297, 305 (sh.). 5; minor product, less polar; m/z 388 (M^+), 324 (M-S_2); δ ($CDCl_3$) 3.92, 3.93, 3.96 (3H each, s), 4.69 (1H, d, 1.2), 5.26, 6.63 (1H each, s), 7.17 (1H, d, 1.2). The three O-methyl signals and the lower chemical shift of H-11 (δ 5.26) of 5 than that of 4 support the enol ether structure 5. The CD spectrum of 4 is very similar to that of 1, which implies that no change occurred in the frame work of the structure of 4 on this methylation.

One-pot reaction of 4 with MeI in pyridine at r.t. followed by SBH reduction in MeOH[12] gave two isomers (6 and 7). 6: m/z 418 (M^+, $C_{16}H_{19}N_2O_5S_2Cl$), 387 (M-OMe), 371 (M-SMe); δ ($CDCl_3$) 2.11, 2.19, 3.10. 3.87, 3.94 (3H each, s), 4.87 (1H, s), 5.10 (1H, d, 1.2), 6.55 (1H, s), 7.22 (1H, d, 1.2). 7: m/z 418 (M^+), 387 (M-OMe), 371 (M-SMe); δ($CDCl_3$) 1.85, 2.43, 3.18, 3.88, 3.90 (3H each, s), 4.71 (1H, s), 4.77 (1H, br. s), 6.61 (1H, s), 7.23 (1H, d, 1.2). Two S-methyl signals (δ 2.11 and 2.19 in 6 and 1.85 and 2.43 in 7 and the carbonyl absorption (1691 cm^{-1})[9] of the mixture show reductive opening with methylation of the disulfide linkage. Each compound reaches to an equilibrium mixture (6 : 7 ≒ 1 : 1.3) in MeOH at r.t. overnight. This facile isomerization is explained by participation of the lone pair electons on the nitrogen carrying a S-methyl group on it to form an ionic intermediate which results in liberation of an anomer at C-8.

Reaction of the mixture 6 and 7 with NaOMe in MeOH yielded a methanol adduct (8): m/z 450 (M^+, $C_{17}H_{23}N_2O_6S_2Cl$), 403 (M-SMe), 371 (M-MeOH-SMe); λ_{max}^{MeOH} nm 230 (sh.), 295; λ_{max}^{+NaOMe} 318 nm. The bathochromic shift (23 nm) in alkaline conditions strongly suggests the presence of a phenolic hydroxyl in 8. The nucleophilic attack of methoxide anion on C-8 produced 8.

It is rather surprising that a compound with such a labile functionarity as thiosulfenic amide occurrs naturally. N-(Thiosulfenyl)phthalimides[13] are, to our knowledge, known synthetic compounds with a similar partial structure.

Further comfirmation of the structure of 1 will be published elsewhere.

Acknowledgements. We thank Prof. S. Abe of Kyoto Prefectural University for the identification of the fungus and Prof. T. Oishi of the Institute of Physical and Chemical Research (IPCR) for helpful discussions. We are greatful to Drs. S. Takeuchi and Y. Kohno of IPCR for their help for FT-NMR measurements. We are also indebted to Mr. Y. Esumi of IPCR for high resolution mass measurements and to Miss H. Tanaka of IPCR for her assistance for the fermentation of the fungus.

References

1) Aspirochlorine (1) has different activity from that of canadensolide. 1 shows not only growth inhibition of mycelium but also enormous numbers of spherical swellings on the hyphae of Phytophthora cinnamomi (A_1 mating type). Isolation and the biological activity of 1 will be published in detail elsewhere.

2) This fungus was isolated from the air and identified by Prof. Shigeo Abe of Faculty of Agriculture, Kyoto Prefectural University, Sakyo-ku, Kyoto 606, Japan.

3) N.J. McCorkinkale, J.L.C. Wright, P.W. Brian, S.M. Clarke and S.A. Hutchinson, Tet. Lett., 727 (1968); M. Kato, R. Tanaka and A. Yoshikoshi, Chem. Comm., 1561 (1971).

4) D.H. Berg, R.P. Massing, M.M. Hoehn, L.D. Boeck and R.L. Hamill, J. Antibiot., 29, 394 (1976).

5) The formulae of the ions in this paper were confirmed by high resolution mass spectrometry.

6) D.H. Berg, R.L. Hamill and M.M. Hoehn, C. A., 86, 41802g (1977): U. S. Patent, 3,991,052 (1976); idem., ibid., 86, 87671s (1977): U. S. Patent, 4,001,086 (1977).

7) An approximate calculation of the chemical shifts of aromatic carbons was made based on the assumption that the substituents at C-1 and C-6 are equivalent to methoxyl and ethyl groups, respectively, using a table in the text: E. Breitmair and W. Voelter, "Methods and Application in Organic Chemistry, Vol. 5,C-13 NMR Spectroscopy Monographs in Modern Chemistry," Verlag Chemie Weinheim, New York, 1978, p. 213.

8) J.W. Ronaldson, Aust. J. Chem., 29, 2307 (1976); D. Brewer, A.G. McInnes, D.G. Smith, A. Taylor and J.A Walter, J. Chem. Soc., Perkin I, 1248 (1978); G. Deffeux, M.A. Baute, R. Baute and M.J. Filleau, J. Antibiot., 31, 1102 (1978).

9) K.H. Michel, M.O. Channey, N.D. Jones, M.M. Hoehn and R. Nagarajan, J. Antibiot., 27, 57 (1974); S. Safe and A. Taylor, J. Chem. Soc. (C), 1189 (1971).

10) K.C. Murdock, J. Med. Chem., 17, 827 (1974).

11) H. Budzikiewicz, C. Djerassi and D.H. Williams, "Structure Elucidation of Natural Products by Mass Spectrometry," Holden Day, San Francisco, 1964, Vol. 2, p. 249.

12) W.D. Jamison, R. Rahman and A. Taylor, J. Chem. Soc. (C), 1564 (1969).

13) A.B. Sulivan and K. Bostany, Int. J. Sulfur Chem., A, 1, 207 (1971).

(Received in Japan 11 January 1982)