Tetrahedron Letters,Vol.24,No.16,pp 1703-1706,1983 0040-4039/83/161703-04$03.00/0
Printed in Great Britain

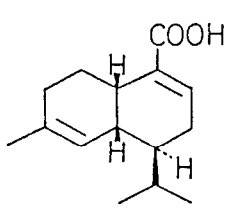
THE REVISED STRUCTURE OF SCLEROSPORIN, A SPOROGENIC SUBSTANCE OF *SCLEROTINIA FRUCTICOLA*. THE TOTAL SYNTHESIS OF (±)-SCLEROSPORIN

Masato Katayama and Shingo Marumo*
Department of Agricultural Chemistry, Nagoya University, Nagoya 464, Japan
Hiroyuki Hattori
National Institute for Basic Biology, Okazaki 444, Japan

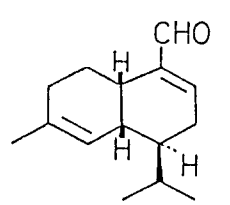
Summary: The structures of sclerosporin and its aldehydic congener, sclerosporal, have been revised as 1 and 2, respectively, on the basis of detailed spectral analysis of sclerosporal with 360 MHz ^{1}H NMR and their total synthesis.

Sclerosporin, a sporogenic substance isolated from *Sclerotinia fructicola*, dramatically induced the formation of asexual arthrospores in this fungal mycelium at a concentration of 1 ng/ml.[1] In our previous study,[2] the structures of sclerosporin and sclerosporal were believed to possess a gaiane skeleton. Evidence for the gaiane skeleton was derived from the close similarity in the mass spectra of the hydrocarbon, $C_{15}H_{28}$, which was chemically derivatized from sclerosporal through four steps of ultramicro-reactions, to those of authentic stereoisomers of guaiane. Since the structures previously postulated were deduced from very small amounts of sclerosporin (182 μg) and sclerosporal (194 μg) on the basis of the limited experimental data, we continued synthetic investigation to confirm the proposed structures. This work revealed that the mass spectrum of *trans*-guaiane[3] was almost indistinguishable from that of *trans*-cadinane[4] (Fig. 1). Thus, our previous conclusion on the carbon skeleton based solely on the mass spectral comparison required reinvestigation.

We wish now to present the correct structures of sclerosporin and sclerosporal as 1 and 2, respectively, which were deduced by detailed spectral analysis of sclerosporal with 360 MHz ^{1}H NMR. This has now been confirmed unambigously by the total synthesis of both compounds.



1



2

In order to obtain enough sample for spectral analysis, the fungus was cultured repeatedly on agar media. From 5050 Petri dishes (101 liters) 3.8 mg of pure sclerosporal was isolated. The detailed ^{1}H NMR analysis of sclerosporal with extensive spin-decoupling experiments,

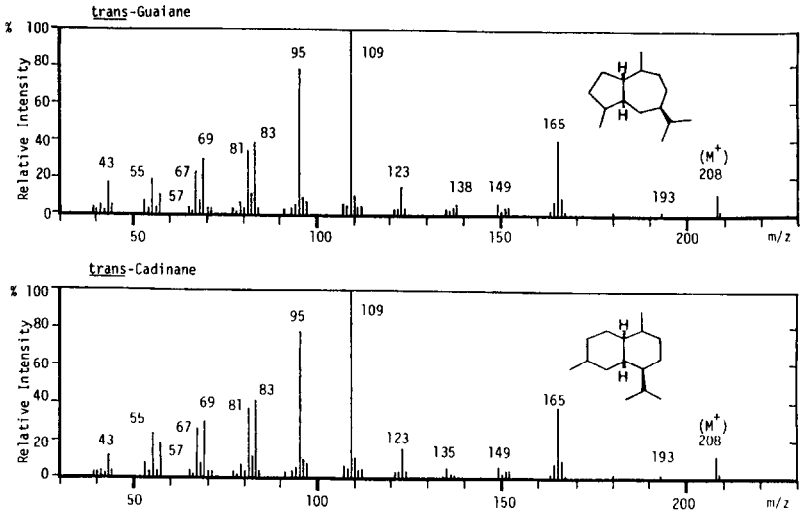
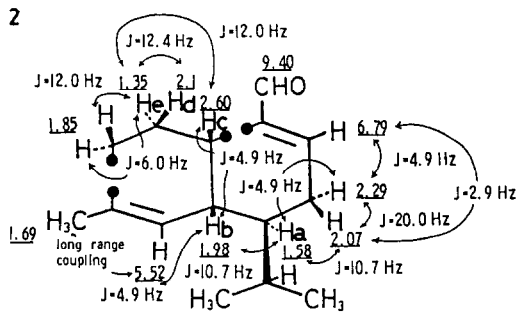
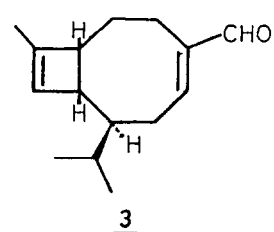


Fig. 1. Mass spectra of *trans*-guaiane and *trans*-cadinane

revealed the relationship of all twenty-two protons, as shown in Fig. 2. Namely, a downfield-shifted vinyl proton at δ 6.79 (1H, dd, J=4.9, 2.9 Hz), located at β-position of the α,β-unsaturated aldehyde, was coupled to methylene protons at δ 2.29 (1H, dt, J=20.0, 4.9 Hz) and 2.07 (1H, ddd, J=20.0, 10.7, 2.9 Hz), respectively. These methylene protons were coupled to a methine proton (Ha) at δ 1.58 (1H, tt, J=10.7, 4.9 Hz), which was further coupled to a methine proton (Hb) at δ 1.98 (1H, dt, J=10.7, 4.9 Hz). Hb had two more adjacent protons at δ 5.52 (vinyl H, broad s, $W_{1/2}$=9 Hz) and at δ 2.60 (Hc, broad d, J=12.0 Hz). Irradiation of this vinyl proton sharpened a methyl signal at δ 1.69 (3H, s), and that of Hc revealed that it was coupled to methylene protons of He at δ 1.35 (ddt, J=12.4, 12.0, 6.0 Hz) and Hd at δ 2.1 (m). Although all coupling constants of Hd could not be elucidated, its signal shape as well as those of He indicated that they were coupled to adjacent methylene protons (δ 1.85). An addition of $Eu(fod)_3$ (reagent/substrate=0.3 molar ratio, $CDCl_3$) deshielded the Ha signal from δ 1.58 to 1.94, under these conditions, Ha was shown to be further coupled to a doublet-septet methine proton of an isopropyl group (two methyls at δ 1.04 and 1.10 (each d, J=7.0 Hz)).[5] The relative stereochemistry of Ha and Hb, and of Hb and Hc, was *trans* and *cis*, respectively, from their J values, 10.7 and 4.9 Hz.

Fig. 2

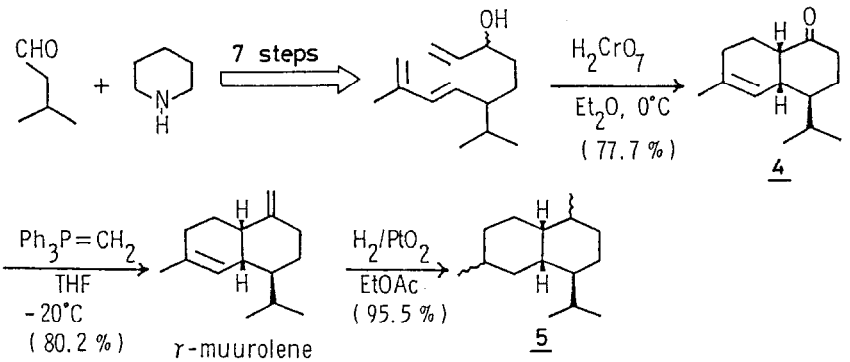




3

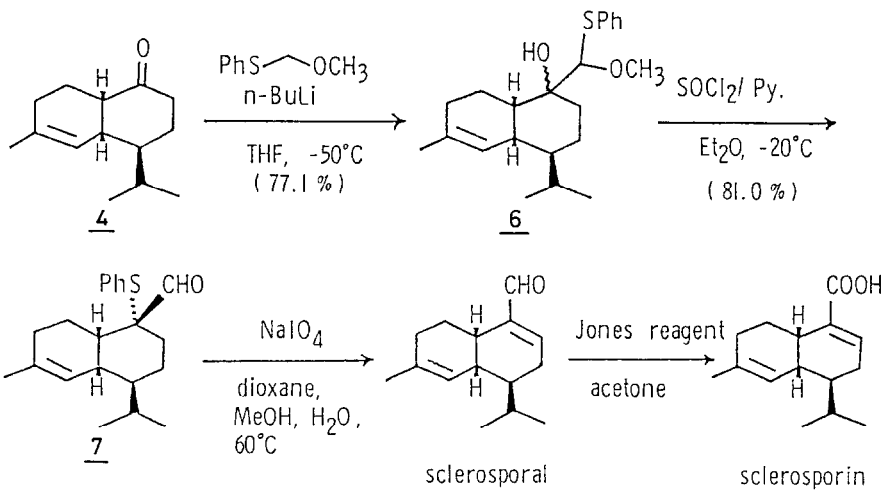
The consecutive spin-decoupling experiments deduced two possible structures, 2 and 3, for sclerosporal. The structure 2 to be more reasonable than 3 was obtained from GLC and spectral comparison of *cis*-cadinane with the saturated hydrocarbon obtained by catalytic hydrogenation of sclerosporene ($C_{15}H_{24}$), another metabolic product of this fungus, already known to have the same carbon skeleton as sclerosporal.[2] Namely, the stereoisomeric mixture of *cis*-cadinane (5), prepared from *cis*-ketonic compound (4) synthesized by Taber's method[6] (Scheme 1), showed four peaks in capillary GLC,[7] whose retention times agreed well with those of the saturated hydrocarbon, and the mass spectra of both compounds were also superimposable. The partial structure surrounding the aldehydic group was supported by the larger downfield-shifts of Hc, Hd and He, upon addition of $Eu(fod)_3$, compared with those of other protons in 2.

Scheme 1



In order to confirm the proposed structures of 2 and 1, both sclerosporal and sclerosporin were synthesized, as shown in Scheme 2, from *cis*-ketone (4), whose relative stereochemistry was established unambigously. 4 was reacted with an anion of methoxymethyl phenyl sulfide (prepared with 1.05 equiv. *n*-butyllithium)[8] to afford the 2:1 mixture of the epimeric alcohol (6).[9] 6 was, without separation of the isomers, treated with thionyl chloride-pyridine, producing the rearranged phenylthio aldehyde (7)[10] as a sole product. Oxidation of the sulfide

Scheme 2



(7) with sodium metaperiodate followed by elimination of sulfenic acid gave racemic sclerosporal, whose ^{1}H NMR (100 MHz) and mass spectra as well as GLC and TLC behavior were identical with those of natural sclerosporal. Oxidation of sclerosporal with Jones reagent afforded racemic sclerosporin, which was identical in a comparison of spectral (^{1}H NMR and mass) data and Rf values on TLC with those of natural sclerosporin. Thus, the structures of sclerosporin and sclerosporal were established, except for the absolute stereochemistry. Synthetic sclerosporin induced the abundant production of asexual arthrospores at a concentration of 1 μg/ml in the mycelium under non-sporulatable, light-grown conditions. Thus, the structure of sclerosporin was further supported by biological activity. The absolute stereochemistry of sclerosporin is now under investigation.

Acknowledgment: The authors are grateful to Professor Kenji Mori of the University of Tokyo for his generous gift of synthetic samples of the incorrect structures of sclerosporin and sclerosporene. This work was supported in part by scientific grant from the Ministry of Education, Science and Culture, Japan.

References and Notes

1) M. Katayama and S. Marumo, Agric. Biol. Chem., 42, 505 (1978).

2) M. Katayama and S. Marumo, Tetrahedron Letters, 1773 (1979).

3) The sample of *trans*-guaiane was prepared by catalytic hydrogenation (PtO_2, EtOAc) of *trans*-guaia-3,10(15)-diene synthesized by Professor K. Mori, the University of Tokyo.

4) The sample of *trans*-cadinane was prepared by catalytic hydrogenation of ε-cadinene synthesized by the methods of Bardhan and Mukherji and of Rao and Dev. J. C. Bardhan and D. N. Mukherji, J. Chem. Soc., 4629 (1956). M. V. R. K. Rao, G. S. K. Rao and S. Dev, Tetrahedron Letters, 27 (1960); Tetrahedron, 22, 1977 (1966).

5) Without addition of $Eu(fod)_3$, the isopropyl signals appeared at δ 0.95 (3H, d, J=7.3 Hz), 0.86 (3H, d, J=6.8 Hz) and 2.1 (1H, m), respectively.

6) D. F. Taber and B. P. Gunn, J. Am. Chem. Soc., 101, 3992 (1979).

7) GLC analysis with Shimadzu GC-6AM gas chromatograph on a capillary column (30 m SCOT, PEG 20M, column temp. 110 --> 150°C, 5°C/min) showed the stereoisomeric mixture of *cis*-cadinane appearing as four peaks at Rts 28.1, 28.7, 30.0 and 30.6 min, respectively. GLC of both synthetic and natural compounds revealed that they had the same four peaks, as ascertained by co-injection experiments.

8) Methoxymethyl phenyl sulfide was prepared by the phase-transfer catalyzed condensation of thiophenol with chloromethyl methyl ether. B. M. Trost and C. H. Miller, J. Am. Chem. Soc, 97, 7182 (1975); M. Lissel and J. Weiffen, Synthetic Communication, 11, 545 (1981); Ae. de Groot and B. J. M. Jansen, Tetrahedron Letters, 22, 887 (1981).

9) 6 was an epimeric mixture, as shown by its ^{1}H NMR ($CDCl_3$) δ 0.71 (d, J=6.8 Hz, $CH(CH_3)_2$), 0.85 (d, J=6.8 Hz, $CH(CH_3)_2$), 0.87 (d, J=7.0 Hz, $CH(CH_3)_2$), 1.65 (3H, s), 3.47 (s, OMe), 3.48 (s, OMe), 4.97 (s, CH-OMe), 5.45 (broad s, vinyl H), 7.05-7.65 (5H, phenyl protons).

10) 7: ^{1}H NMR ($CDCl_3$) δ0.93 (3H, d, J=6.8 Hz), 0.96 (3H, d, J=6.8 Hz), 1.66(3H, s), 1.2-2.2 (11H), 2.56(1H, broad s), 5.61 (1H, broad d, J=5.6 Hz), 7.05-7.45 (5H, m), 9.34 (1H, s).

(Received in Japan 15 November 1982)