CHEMISTRY LETTERS, pp. 693 - 694, 1984. © The Chemical Society of Japan 1984

STRUCTURE OF TWO STRESS METABOLITES, SPIROBROUSSONIN A AND B, FROM DISEASED PAPER MULBERRY[1)]

Mitsuo TAKASUGI,* Nobuyuki NIINO, Masaki ANETAI, Tadashi MASAMUNE,
Akira SHIRATA,† and Kokichi TAKAHASHI†
Department of Chemistry, Faculty of Science, Hokkaido University,
Sapporo 060
†Sericultural Experiment Station, Yatabe-machi, Tsukuba-gun 305

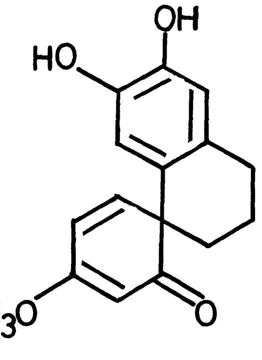
The fungus-inoculated *Broussonetia papyrifera* shoot cortical tissues produced two stress metabolites named spirobroussonin A and B. Structure elucidation of these compounds is described.

In the preceding paper[1)] we reported the isolation and structure elucidation of eight minor phytoalexins from paper mulberry (*Broussonetia papyrifera* Vent.), which had been inoculated with *Fusarium solani* f. sp. *mori*. Further examination of polar fractions of the dichloromethane-methanol (97:3) eluates[1)] led to isolation of two new compounds with weak antifungal activity,[2)] named spirobroussonin A (**1**) and B (**2**), in 0.027 and 0.0064% yields (from the dried tissues), respectively. We report herein the structure determination of these stress metabolites.

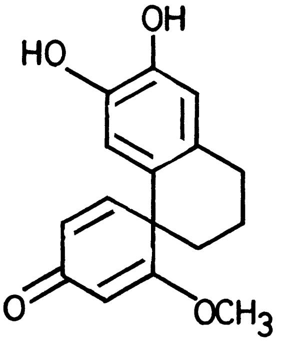
Spirobroussonin A (**1**), mp 253-254 °C, $[\alpha]_D$ 0°, had molecular formula $C_{16}H_{16}O_4$ [m/z 272.1032 (M^+)], gave its diacetate[3)] (**1a**) and its dimethyl ether[3)] (**1b**) (CH_2N_2 in ether-EtOH), mp 120-122 °C, and exhibited the following spectra: UV (EtOH), 310 nm (ε 3980, sh), 294 (6450, sh), 288 (6720), 240 (7140, sh), and 211 (40300); IR (KBr), 3450, 1650, 1585, and 1548 cm^{-1}. The ^1H NMR spectrum (CD_3SOCD_3) revealed the presence of one methoxyl and two phenolic hydroxyl groups [δ 3.81 (3H, s) and 8.60 (2H, br s, 2OH)], a trimethylene moiety [δ 1.8 - 2.1 (4H, m) and 2.56 (2H, m)], and five protons [δ 6.05 and 6.45 (each 1H, s); δ 5.43 (1H, d, J = 2.1 Hz), 6.12 (1H, dd, J = 10.0 and 2.1), and 6.59 (1H, d, J = 10.0)], while the ^{13}C NMR spectrum (CD_3SOCD_3) indicated that **1** consisted of five sp^3-type carbon atoms with one spiro one [δ 18.0, 28.2, and 33.4 (each t), 52.2 (s), and 56.0 (q)] and eleven sp^2-type carbon atoms with one carbonyl one [δ 98.5, 114.2, 115.8, 117.1, and 149.4 (each d), 126.1, 127.5, 143.4, 144.3, 170.5, and 202.3 (each s)]. These spectral data, coupled with a positive color reaction of **1** with sodium molybdate(VI) (vicinal location of two phenolic hydroxyl groups), suggested the presence a 4,5-disubstituted catechol moiety and a 2,4-cyclohexadienone ring containing the spiro carbon atom. In view of the co-occurrence of broussonin E[1)] (**3**), formula **1** could be assigned to this compound. This was confirmed by the following synthesis of **1b**: compound **3**, prepared from 2',3-dihydroxy-4,4'-dimethoxychalcone [i, H_2/Pd-C in ether (90%), and ii, Zn-Hg/HCl (82%)], was adsorbed on Amberlite XAD-2 resin and treated with aqueous iron(III) chloride (0 °C, 12 h). Extraction of the resin with ether gave an oxidative coupling product,[3)] mp 197-198 °C, $C_{17}H_{18}O_4$ [m/z 286.1202 (M^+)], in 36% yield. This compound was converted into its methyl ether, mp 122-124 °C, which was identical with **1b**, derived from natural one in all respects (MS, UV, IR, NMR).

Spirobroussonin B (**2**), mp 232-233 °C, $[\alpha]_D$ 0°, had the same molecular formula $C_{16}H_{16}O_4$ [m/z 272.1054 (M^+)] as **1**, and gave its diacetate[3)] (**2a**), amorphous, and its dimethyl ether[3)] (**2b**), mp 136-138 °C. The ^1H [δ 1.8 - 2.0 (4H, m), 2.61 (2H, m), 6.17 and 6.48 (each 1H, s), and 8.74 (2H, br s, 2OH)] and ^{13}C NMR spectra [δ 19.0, 28.2, and 33.1 (each t), 113.6 and 116.1 (each d), 123.9, 127.7, 143.7, and 144.5 (each s)] indicated the presence of the same 6,7-dihydroxytetralin moiety as **1**, while the UV [260 nm (ε 9300, sh), 230 (17900), and 210 (36800)], IR (3300, 1654, and 1570 cm^{-1}), and ^1H [δ 3.63 (3H, s), 5.65 (1H, d, J = 1.5 Hz), 5.94 (1H, dd, J = 10.0 and 1.5), and 6.89 (1H, d, J = 10.0)] and ^{13}C NMR spectra [δ 55.8 (q), 101.9, 122.9, and 151.8 (each d), 45.9 (s), 179.9, and 186.9 (each s)] strongly suggested that **2** would involve a 2,5-cyclohexadienone ring with the spiro carbon atom instead of the 2,4-cyclohexadienone moiety in **1**. These spectral data, combined with a positive color reaction of **2** with sodium molybdate(VI), led to assignment of formula **2** to this compound, which was confirmed by the following synthesis: a 1,3-diphenylpropane (**4**), prepared by two-step reduction of 4'-benzyloxy-3-hydroxy-4,2'-dimethoxychalcone [i, $LiAlH_4$-$AlCl_3$ in ether (44%), and ii, H_2/Pd-C in EtOH (42%)], was oxidized with the complex $[Fe(DMF)_3Cl_2][FeCl_4]$[4)] in ether-water gave an oxidative coupling product,[3)] mp 202-203 °C, $C_{17}H_{18}O_4$[m/z 286.1182 (M^+)], in 75% yield. This compound was converted into its methyl ether, mp 137-139 °C, which was identical with **2b**, derived from natural one in all respects (MS, UV, IR, NMR).

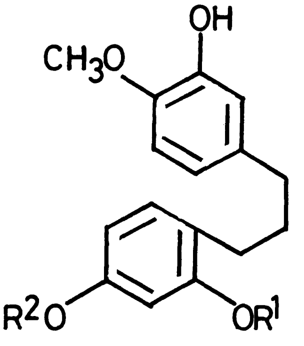
It is to be noted that these spirobroussonins possess no optical activity (linear ORD curve), although these compounds would be formed by *in vivo* oxidation of broussonins[1)] with a 1,3-diphenylpropane skeleton.



1



2



3 R^1=H, R^2=CH_3
4 R^1=CH_3, R^2=H

References

1) Part 14 of the series "Studies on Phytoalexins of the Moraceae;" Part 13, M. Takasugi, N. Niino, S. Nagao, M. Anetai, T. Masamune, A. Shirata, and K. Takahashi, Chem. Lett., (the preceding paper).
2) Antifungal activity of **1** and **2** could not be examined exactly owing to the low solubility in solvents.
3) All new compounds gave satisfactory spectral data (MS, HR-MS, UV, IR, and ^1H and/or ^{13}C NMR).
4) S. Tobinaga and E. Kotani, J. Am. Chem. Soc., 94, 309 (1972).

(Received February 8, 1984)