Journal of Natural Products
Vol. 53, No. 5, pp. 1345-1346, Sep-Oct 1990

A NEW DIHYDROSTILBENE FROM DISEASED *DIOSCOREA MANGENOTIANA*

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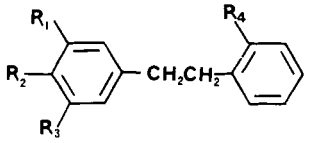
ABSTRACT.—*Dioscorea mangenstiana* tuber infected with *Botryodiplodia theobromae* gave β-sitosterol, dihydropinosylvin, and a 4,2′-dihydroxy-3,5-dimethoxydihydrostilbene as its major inducible constituents in the ethylacetate extract.

In earlier communications (1–3), we described the isolation and characterization of dihydrostilbene phytoalexins from root tubers of *Dioscorea rotundata, Dioscorea alata,* and *Dioscorea dumentorum* and bulbils of *Dioscorea bulbifera* infected with *Botryodiplodia theobromae* Pat., a fungal pathogen of yams. We have now completed the study of another related plant, *Dioscorea mangenotiana* Meige. (Dioscoreaceae), from which 4,2′-dihydroxy-3,5-dimethoxydihydrostilbene [**2**] was isolated from the infected tubers as the new major metabolite together with the common dihydropinosylvin [**1**]. In addition, β-sitosterol was isolated.

EtOAc extraction of the infected tubers gave an extract which showed two extra reddish spots not found in the controls on Si gel tlc [$CHCl_3$-MeOH (24:1)] detected with vanillin in H_2SO_4. Cc followed by preparative tlc gave **1** and **2**. β-Sitosterol that precipitated from earlier column fractions was also collected and identified (Liebermann-Burchard test, uv, eims, ^1H nmr, identical chromatographic behavior with an authentic sample).

Compound **1** had uv absorptions, eims, and ^1H- and ^{13}C-nmr spectra identical to those earlier reported (1,4) for dihydropinosylvin.



1 $R_1=R_3=OH$, $R_2=R_4=H$
2 $R_1=R_3=OMe$, $R_2=R_4=OH$

Compound **2**, obtained as a viscous mass, had uv absorption maxima at 255 sh and 265 and eims with $[M]^+$ ions at *m/z* 274 ($C_{16}H_{18}O_4$). Other diagnostic ions were present at *m/z* 167 (100%), due to a monohydroxy-dimethoxy tropylium ion, and at 107 (14%), due to a monohydroxy tropylium ion typical of a bibenzyl system (1,2,4). The structure of **2** was suggested by its ^1H-nmr data that showed the characteristic four-proton multiplet at δ 2.86, for the $ArCH_2CH_2Ar$ bridge protons, and a six-proton singlet at δ 3.93 for two equivalent methoxyl protons. A two-proton singlet was observed at δ 6.20, and, when compared with the broad three-proton singlet at δ 6.25 for **1**, suggested a substitution at C-4. This δ 6.20 signal was assigned to protons at C-2 and C-6 in line with similar assignments for δ 6.36 (2H) in bibenzyl moscatilin (5), δ 6.27 (2H) for the 3′-analogue of the proposed compound aloifol 1 (6) and δ 6.37 (2H) in batatasin V (7). The H-2′, -4′, -5′, and -6′ protons of the second ring gave signals identical to those observed for demethyl batatasin IV (1,2) at δ 6.82 (2H) and δ 7.10 (2H), indicating the substitution at the C-2′ position as opposed to those reported for the 3′ analogue, aloifol 1, obtained from *Cymbidium aloifolium* (6).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The fresh tubers of wild *D. mangenotiana* were collected near Ile-Ife, Nigeria. Voucher specimens were deposited at the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife. Si gel

GF_{254} was used for tlc, and Si gel mesh 60–230 was used for cc. The following instruments were used: Varian UV visible spectrophotometer, Varian FT-80 NMR spectrometer, SP-300 Pye-Unican Infrared spectrophotometer, and AE1-MS 902 mass spectrometer (VG-Micromass, UK).

EXTRACTION AND PURIFICATION.—In order to induce phytoalexin production, the fresh tubers (11.5 kg) of *D. mangenotiana* were infected with an aqueous suspension of *B. theobromae* (old stock culture collection 1969 supplied by the Department of Microbiology depository: Obafemi Awolowo University, Ile-Ife) mycelia as previously described (1–3). The EtOAc extract (5.6 g) was chromatographed over a column of Si gel (300 g) with *n*-hexane–$CHCl_3$ (4:1) (600 ml), (3:1) (250 ml), (7:3) (750 ml), and $CHCl_3$-MeOH (1:2) (370 ml) collected in 15-ml fractions. Fractions 24–39 gave a white precipitate which was collected and identified as β-sitosterol. Combined fractions 25–93 were rechromatographed over a Si gel column (100 g) with *n*-hexane and *n*-hexane/MeOH mixtures of increasing polarity. Fractions containing **1** and **2** (tlc) were combined and purified by preparative tlc, using $CHCl_3$-MeOH (24:1), to give 20 mg of **1** (R_f 0.4) and 34 mg of **2** (R_f 0.20), respectively. Uv, eims, and ^{1}H-nmr data of **1** were identical with those earlier reported in the literature for dihydropinosylvin (1,4).

4,2′-DIHYDROXY-3,5-DIMETHOXYDIHYDROSTILBENE [**2**].—Uv λ max (MeOH) 255 sh, 265; λ max ($AlCl_3$) no shift; eims (70 eV) *m/z* (% rel. int.) $[M]^+$ 274 (23), 242 (88), 167 (100), 107 (14), 71 (22), 57 (45); ^{1}H nmr ($CDCl_3$, TMS) δ 2.86 (4H, m, Ar-CH_2CH_2-Ar), 3.93 (6H, s, 3-OMe, 5-OMe), 6.20 (2H, s, H-2, H-6), 6.82 (2H, m, H-3′, H-6′), 7.12 (2H, m, H-4′, H-5′), 7.30 (1H, s, exchangeable with D_2O, 4-OH).

ACKNOWLEDGMENTS

NGK is grateful to OAU/STRC for a postgraduate scholarship. We thank Dr. T.A. Olugbade for nmr determinations and Mr. D. Carter, School of Pharmacy, University of London, for ms data. This work was supported by the International Foundation for Science grant no. F/1164-1.

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Received 25 October 1989