Phytochemistry, Vol. 28, No. 3, pp. 773–774, 1989.
Printed in Great Britain.

0031-9422/89 $3.00 + 0.00

DIHYDROSTILBENE PHYTOALEXINS FROM *DIOSCOREA BULBIFERA* AND *D. DUMENTORUM*

S. A. ADESANYA,* S. K. OGUNDANA† and M. F. ROBERTS‡

Department of Pharmacognosy, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria; †Department of Microbiology, Faculty of Science, Obafemi Awolowo University, Ile-Ife, Nigeria; ‡Department of Pharmacognosy, School of Pharmacy, London University, 29/39 Brunswick Square, London WC1N 1AX, U.K.

(*Received* 1 *August* 1988)

Key Word Index—*Dioscorea bulbifera*; *D. dumentorum*; Dioscoreaceae; *Botryodiplodia theobromae*; dihydroresveratrol; dimethylbatatasin IV; phytoalexin.

Abstract—Analysis of the ethyl acetate extract of the bulbil of *Dioscorea bulbifera* and the tuber of *Dioscorea dumentorum* infected with *Botryodiplodia theobromae* gave demethylbatatasin IV and a new dihydrostilbene, 3,5,4′-trihydroxybibenzyl (dihydroresveratrol), respectively, as their major phytoalexins.

INTRODUCTION

Recent reports have shown that dihydrostilbenes may be induced in the storage organs of a number of species within *Dioscorea*, Section *Enantiophyllum*, namely *Dioscorea batatas*, *D. rotundata* and *D. alata* [1–3]. Although the major food yams belong to this section, a few species in other Sections such as *Combilium* (*D. esculenta*), *Lasiophyton* (*D. hispida*, *D. dumentorum*), *Opsophyton* (*D. bulbifera*) and *Macrogynodium* (*D. frigida*) are also edible [4].

In other families, e.g. *Trigonella*, [5, 6] of the Leguminosae, isoflavonoids induced microbially have been found to have some minor taxonomic importance. In some cases varietal differences may also occur as is observed with *Vigna unguiculata* cultivars in respect of the accumulation of phaseollin or 2′-*O*-methylphaseollinisoflavan [7]. Ireland *et al.* [8] have also noted some differences in the occurrence of batatasins in the different sections of the Dioscoreaceae.

In continuation of our work on phytoalexins in *Dioscorea* species, we report two major compounds in infected bulbils and tubers of *D. bulbifera* and *D. dumentorum* respectively, one of which is a novel compound.

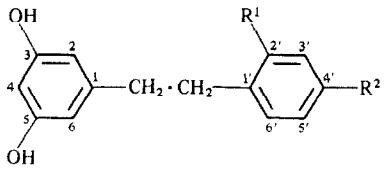
RESULTS AND DISCUSSION

Compounds with antifungal activity were observed in the ethyl acetate (EtOAc) extracts of bulbils of *D. bulbifera* or tubers of *D. dumentorum* infected with *Botryodiplodia theobromae* as a result of using the TLC bioassay with *Cladosporium cladosporoides* as the test organism [9]. Extracts from both *D. bulbifera* and *D. dumentorum* exhibited one major spot (R_f 0.25–0.50 in $CHCl_3$–MeOH 19:1) which inhibited spore germination and also corresponded with a major reddish spot observed when TLC plates were developed and sprayed with vanillin in sulphuric acid. These antifungal compounds were only associated with extracts from infected bulbils and tubers; neither the spots nor the activity were observed with extracts from uninfected plant material.

Compound **1** had an absorbance maximum at 272sh, 275 nm, indicating a bibenzyl system. In the mass spectrum, the M^+ ion at *m/z* 230 ($C_{14}H_{14}O_3$) and the other ions *m/z* 123 (25% dihydroxytropylium ion) and *m/z* 107 (100% monohydroxytropylium ion) are typical of a bibenzyl system [2]. The ^{1}H NMR spectrum contained the characteristic Ar–CH_2–CH_2–Ar four proton multiplet at δ2.85, a proton singlet at δ6.26, and a singlet equivalent to two protons at δ6.30 attributable to a *meta* and two *ortho* protons in the dihydroxybenzyl. These, together with the coupling patterns of the protons at δ6.82 (2H) and δ7.10 (2H) indicated that the structure is 2′,3,5-trihydroxybenzyl. The ^{13}C NMR spectrum confirmed the compound as demethylbatatasin IV previously isolated from *Dioscorea rotundata* [2].

Compound **2** possesses an absorbance maximum at 277 nm which did not show a bathochromic shift with $AlCl_3$ shift reagent, indicating another bibenzyl without an *ortho*-dihydroxy system. The mass spectrum with ions at *m/z* 230 [(M^+)$C_{14}H_{14}O_3$)] and *m/z* 123 (23%) and *m/z* 107 (100%) indicated a close similarity with **1**, in which the respective tropylium ions suggest a benzyl ring with two hydroxyl groups and another with one hydroxyl group. The ^{1}H NMR spectrum also had the characteristic four proton Ar–CH_2–CH_2–Ar signal at δ2.70 and a broad singlet at δ6.21 attributable to three nearly equivalent protons on the dihydroxybenzyl ring, indicating that the third hydroxyl function is not in the 2′ position, since in the spectrum of **1**, these protons are located by two signals [2]. The assignment of this hydroxyl group to the second benzyl ring at the *para* (4′) rather than *meta* (3′) position is defined by the *ortho* and *meta* coupling pattern of two identical signals at δ6.51 and δ6.94, each integrating for two protons. The ^{13}C NMR spectrum with SEMUT showed the expected downfield shift for the C-4′ carbon (29 ppm) compared with the unsubstituted compound, dihydropinosylvin [2]. An upfield shift of the 3′ and 5′ (13 ppm) and a slight downfield shift of the 2′ and 6′ carbons (1 ppm) are also observed [2]. This is consistent

*Author to whom correspondence should be addressed.



1 R^1 = OH, R^2 = H
2 R^1 = H, R^2 = OH

with the structure being 3,5,4′-trihydroxybibenzyl **2**, which is a new natural product and has been named dihydroresveratrol.

Antimicrobial activity

The antifungal and antibacterial activity of **1** has been reported elsewhere [2]. When similarly tested against germination of fungi, **2** was active against *Cladosporium cladosporoides* (MIC 50 μg/ml) and *Trichophyton mentayophytes* (MIC 1000 μg/ml but was not active against *Candida albicans*, *Botryodiplodia theobromae*, *Aspergillus niger* and *A. flavus*. The antibacterial tests indicate activity against *Escherichia coli* NCIB 86 (MIC 1000 μg/ml), *Serratia marcescens* NCIB 1377 (MIC 50 μg/ml) and *Staphylococcus aureus* NCIB 8588 (MIC 50 μg/ml) but no activity against *Klebsiella pneumoniae*, *Baccillus subtilis*, *Pseudomonas aerugenosa* or *Proteus vulgaris* was observed.

Dihydroresveratrol, **2**, did not show a marked antimicrobial activity and in this respect is similar to demethylbatatasin IV, **1** [2]. It is not immediately obvious from the foregoing results that **1** and **2** have a role in the prevention of microbial invasion despite the fact that their production is elicited by fungal infection. However, they may act in other as yet undefined roles, since resveratrol inactivates enzymes with sulphydryl groups [10, 11]. These compounds may also act as precursors of other compounds with better defined antimicrobial activity [2, 12].

The variations in dihydrostilbene phytoalexins produced in *Dioscorea* species in response to microbial attack are of some interest from a chemotaxonomic point of view. The major phytoalexin in *D. batatas* [1], *D. rotundata* [2] and *D. alata* [3] in *Dioscorea*, Section *Enantiophyllum*, is dihydropinosylvin. This compound is not present in *D. bulbifera* in Section *Opsophyton* and *D. dumentorum* in Section *Lasiophyton*. However, demethylbatatasin IV located as the major phytoalexin in *D. bulbifera* also accumulates in *D. alata* and *D. rotundata* [2] while the analogue **2** accumulates in *D. dumentorum*.

EXPERIMENTAL

Wild bulbils of *D. bulbifera* L. and cultivated tubers of *D. dumentorum* (Kunth) Pax. were collected around Ile-Ife. Herbarium and voucher specimen were deposited at the herbarium of the Department of Pharmacognosy, Obafemi Awolowo University, Ile-Ife, Nigeria, where they were identified. The plant materials were induced with an aq. suspension of *B. theobromae* mycelia. Large scale extraction with EtOAc and TLC bioassay to locate antifungal zones with *C. cladosporoides* were performed as described in ref. [2].

D. bulbifera. The EtOAc extract from infected bulbils of *D. bulbifera* (3 g) was subjected to CC using silica gel (mesh 60–120) and eluted successively with C_6H_6–EtOAc–hexane, 3:5:2 (1100 ml) followed by benzene–EtOAc, 5:1. Fractions collected (50 ml) were monitored by TLC using $CHCl_3$ as a solvent system. Fractions 26–53 were bulked and subjected to repeated prep TLC in $CHCl_3$, followed by $CHCl_3$–MeOH, 19:1 to isolate compound **1**, the major reddish spot in the extract.

Demethylbatatasin IV, **1**. Yield 94 μg/g fresh weight; absorbance maxima, nm: 267, 272sh, 282; $+AlCl_3$: no shift. EIMS, ^1H NMR, ^{13}C NMR characteristics were identical with data given in the literature [2].

D. dumentorum. The EtOAc extract from an infected tuber of *D. dumentorum* (40 g) was subjected to CC using silica gel (mesh 60–120) and eluted with C_6H_6–EtOAc, 3:1 (4000 ml), followed by C_6H_6–EtOAc, 2:1. Fractions (100 ml) were collected and monitored with TLC C_6H_6–EtOAc, (2:1). Fractions 3–4 gave A (18 g); 5–24, B (3 g); 25–43, C (2 g); and 44–55, D (8 g). The antifungal zone and the reddish spot was located in B which was subjected to silica gel prep TLC ($CHCl_3$–MeOH, 19:1) to purify dihydroresveratrol, **2**.

Dihydroresveratrol, **2**. Yield 200 μg/g fresh weight. Absorbance maxima, nm: 277; $+AlCl_3$: no shift; + NaOMe: 291. MS m/z (rel. int.): 230 (15) $[M]^+$; 122 (10); 123 (25), 107 (100). ^1H NMR, 100 MHz ($CDCl_3$ + TMS): δ2.71 (4H, *br s*, Ar–CH_2–CH_2–Ar), 6.21 (3H, *br s*, H-2, H-4, H-6), 6.51 (2H, *dd*, J = 8.0, 1.8 Hz, H-3′, H-5′), 6.94 (2H, J = 8.0, 1.8 Hz H-2′, H-6′). ^{13}C NMR ($CDCl_3$ + a drop CD_3OD + TMS): δ36.69 (Ar–CH_2–), 38.20 (–CH_2–Ar), 100.63 (C-4), 107.82 (C-2, C-6), 115.39 (C-3′, C-5′), 129.64 (C-2′, C-6′), 133.62 (C-1′), 145.01 (C-1) 154.57 (C-4′), 157.62 (C-3, C-5).

Antimicrobial assay. The fungal and bacterial strains were maintained as described in literature [2]. The inhibition of fungal germination was determined by placing 0.1 ml of each concn of **2** (50, 100, 250, 1000 μg/ml) in sterile H_2O in different 6 mm wells in an agar plate (triplicates). A plug of fungal mycelia was placed at the centre and then incubated for 48 hr at 25° before measuring the zones of inhibition. The minimum inhibitory concentration (MIC) was taken as the lowest concentration showing inhibitory zones. The same method was followed for the antibacterial tests except that the agar plate was seeded with the test bacteria before the holes were made. The results were taken after 24 hr incubation.

Acknowledgements—We thank Dr. T. O. Olugbade (Department of Pharmaceutical Chemistry, Obafemi Awolowo University) for NMR determinations on the Varian FT80 spectrometer. The work was supported by International Foundation for Science grant No. F/1164-1.

REFERENCES

1. Takasugi, M., Kawashima, S., Monde, K., Katsui, N., Masamune, T. and Shirata, A. (1987) *Phytochemistry* **26**, 371.
2. Fagboun, D. E., Ogundana, S. K., Adesanya, S. A. and Roberts, M. F. (1987) *Phytochemistry* **26**, 3187.
3. Cline , E. I., Adesanya, S. A., Ogundana, S. K. and Roberts, M. F. (1988) *Physiol. Mol. Plant Pathol.* (in press).
4. Cursey, D. G. (ed.) (1967) in *Yams*, p. 31. Longmans, London.
5. Ingham, J. L. and Harborne, J. B. (1976) *Nature* **260**, 241.
6. Ingham, J. L. (1981) *Biochem. Syst. Ecol.* **9**, 275.
7. Preston, W. M. (1975) *Phytochemistry* **14**, 1131.
8. Ireland, C. R., Schwabe, W. W. and Cursey, D. G. (1981) *Phytochemistry* **20**, 1569.
9. Coxon, D. T., Ogundana, S. K. and Dennis, C. (1982) *Phytochemistry* **21**, 1389.
10. Ingham, J. L. (1976) *Phytochemistry* **15**, 1791.
11. Lyr, H. (1961) *Enzymologia* **23**, 231.
12. Stoessl, A. (1982) in *Phytoalexins* (Bailey, J. A., and Mansfield, J. W., eds), p. 155. Blackie, Glasgow.