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A NATURALLY-OCCURRING ANALOGUE OF PHASEOLOTOXIN (BEAN HALOBLIGHT TOXIN)

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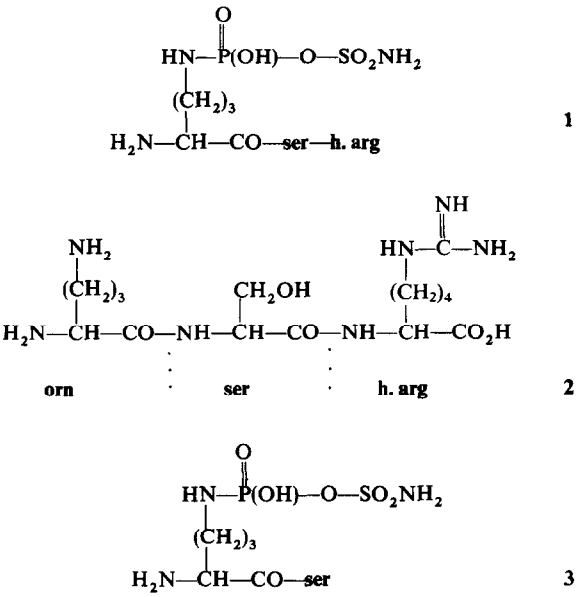
The name phaseolotoxin has been given to a biologically-active compound produced in liquid cultures of the bean halo-blight bacterium *Pseudomonas phaseolicola* [1]. Purified phaseolotoxin causes several of the characteristic symptoms [2] of the disease on bean: chlorosis of primary leaves and subsequent chlorosis in trifoliate leaves resulting from systemic movement of toxin, suppression of leaf expansion, particularly in systemically-affected trifoliate leaves, and accumulation of ornithine in the affected tissue. Despite the low concentration of phaseolotoxin formed in the culture medium (*ca* 1 mg/l), the final purified product accounted for at least 80% of the chlorosis-inducing capability of the original, crude, cell-free culture medium. Thus there are reasonable indications that phaseolotoxin is the active principle or phytotoxin that causes the major symptoms of bean haloblight.

The structure of phaseolotoxin was found to be (N^{δ}-phosphosulphamyl) ornithylalanylhomoarginine [1, 3]. During the course of our studies, some results indicated that minor amounts of compounds related to phaseolotoxin might also be present in the crude extract. This account reports the isolation and characterization of one such compound which shows the same biological effects on beans as does phaseolotoxin.

This second toxin was detected during the final step in the purification of 43 mg of ^{35}S-labelled phaseolotoxin by procedures already described [1]. At this step the radioactivity of fractions collected from a QAE Sephadex column was monitored, and ^{35}S-toxin was found in four consecutive fractions with a 2:51:43:4 distribution of radioactivity. Each fraction was examined by 2D thin-layer electrophoresis and chromatography (TLE/TLC). In the first three fractions phaseolotoxin was the only ninhydrin-reacting and ^{35}S-containing compound detected. In the final fraction a second compound (**1**) was detected. It contained *ca* 50% of the radioactivity in the fraction and reacted with ninhydrin giving an orange colour similar to that obtained with phaseolotoxin, had the same TLE mobility but was chromatographically slower moving. Because it was similar to phaseolotoxin we examined it in more detail*.

The fraction containing phaseolotoxin and **1** was subjected to preparative-TLC in BuOH–HOAc–H_2O–pyr (5:1:4:4), and the band (R_f 0.15) containing **1**, located by autoradiography, was eluted with H_2O. The resulting extract was further purified by ion-exchange chromatography on QAE Sephadex [1]. The final product was homogeneous as determined by 2D TLE/TLC, and no phaseolotoxin was present.



Scheme 1.

Compound **1** contained both P (molybdate spray) and S (^{35}S incorporation). Strong acid hydrolysis (6M HCl, 100°, 16 hr) liberated three ninhydrin-reacting products in *ca* equimolar amounts. These were identified as ornithine, serine and homoarginine by TLE/TLC procedures [1].

More gentle acid hydrolysis (50 mM HCl, 40°, 2 hr) yielded a single ninhydrin-reacting product (**2**), distinct from **1**, and this on strong acid hydrolysis was shown to contain the same amino acids as **1**.

The reaction of **1** with carboxypeptidase B (CPB) and leucine aminopeptidase (LAP) [1] gave useful information about its structure. CPB released a single amino acid, homoarginine, showing this to be the C-terminal amino acid. The other product of the CPB reaction, **3**, differed on 2D TLE/TLC (slower-moving chromatographically) from the corresponding product obtained by the action of CPB on phaseolotoxin. In contrast, the

* In a new preparation of the ^{35}S-labelled toxin-containing fraction, the proportion of compound **1** to phaseolotoxin was 1:16 by ^{35}S radioactivity measurement.

product of the LAP reaction (apart from amino acid products, serine and homoarginine) was identical to the corresponding product obtained by the action of LAP on phaseolotoxin: thus it is N^{δ}-phosphosulphamyl ornithine. Standard *N*-terminal analysis with 2,4-dinitrofluorobenzene showed that the ornithyl residue of **1** was *N*-terminal and δ-substituted.

Together these data supply sufficient information to establish the structure of compound **1**. In the peptide portion, ornithine is *N*-terminal and homoarginine is *C*-terminal, so that the amino acid sequence must be ornithine-serine-homoarginine. This is as in phaseolotoxin, but with serine replacing alanine. The ornithine is N^{δ}-substituted, and the substituent group is sulphamyl phosphate. Thus **1** is (N^{δ}-phosphosulphamyl) ornithithyl-serylhomoarginine (**1**); compound **2** is ornithylseryl-homoarginine (**2**) and compound **3** is (N^{δ}-phosphosulphamyl) ornithylserine (**3**).

The new toxin is thus a serine analogue of phaseolotoxin. There is good evidence that tabtoxin, the toxin of *Ps. tabaci* and other *Pseudomonas* species, also has a serine analogue, and the name [2-serine]-tabtoxin has already been given to this compound [4, 5]. In keeping with this useful terminology we propose the name [2-serine]-phaseolotoxin for the minor toxin of *Ps. phaseolicola*.

EXPERIMENTAL

All procedures used have been previously described [1].

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2-METHYL-3-BUTEN-2-YL-β-D-GLUCOPYRANOSIDE FROM *FERULA LOSCOSII*

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Ferula loscosii (Lge.) Wk. (*Elaeoselinum loscosii* Lge.)* is an endemic species in Spain (near Aranjuez and Chiprana, Teruel). From the aerial parts of this plant a new glucoside, $C_{11}H_{20}O_6$, (**1**) (0.8% of dry plant) has been isolated. Acid hydrolysis of **1** gave D-glucose and treatment of **1** with Ac_2O–Py afforded a tetraacetyl derivative (**2**). The 100 MHz NMR spectrum of **1** showed signals at δ 6.28 (1H), 5.28 (1H) and 5.03 (1H) for a $CH_2{=}CH{-}\overset{|}{\underset{|}{C}}{-}$ grouping; 1.43 (3H) and 1.37 (3H) for $(Me)_2C<$ and the expected signals for the D-glucopyranose moity between 4.78–3.50. The anomeric proton appeared as a doublet at 4.78 (J 8.0 Hz) indicative of β-configuration at the anomeric centre. The above data suggest formula **1** for this compound. On the other hand, hydrogenation of **1** gave **3**, a dihydro derivative, the NMR spectrum of which showed signals for Me–CH_2– at 0.95 and 1.62. The tetraacetyl derivative of **3** (compound **4**) was identical in all respects with the product obtained by a modified Koenigs–Knorr reaction [1] of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide with 2-methyl-3-buten-2-ol.

*J. Borja does not agree with J. F. M. Cannon, (1968) *Flora Europeae* Vol. 2 p. 359 (Cambridge University Press) who identifies *Ferula loscosii* with *F. communis* L., since the former considers it is a particular species. (Personal communication Dr. J. Borja, Instituto Botánico A. J. Cavanilles, Madrid).

EXPERIMENTAL

Mp's are uncorrected. NMR spectra were recorded at 100 MHz. Plants were collected at Aranjuez (Madrid) in June 1974. Identified by Dr. J. Borja, Voucher specimens (No 90066) were deposited in the Herbarium, Faculty of Pharmacy (Ciudad Universitaria, Madrid).

Extraction and isolation of **1**. Dry aerial parts (800 g) were extracted first with ether-petrol (1:2) and then with MeOH in a Soxhlet. The methanolic extract (40 g) was introduced in a drypacked column of Si gel. Elution with $CHCl_3$ and $CHCl_3$–MeOH (100:3) gave crude **1** (10 g), rechromatography yielded pure **1** (6.5 g): mp 135–136° (Me_2CO); $[\alpha]_D^{20°}$ −25° (*c* 1.07; Py); ν_{max}^{nujol} 3450 cm^{-1}; NMR (Py): δ 6.28 (H-3, *q*, $J_{3,4_1}$ 10.0, $J_{3,4_2}$ 18.0 Hz), 5.28 (H-4_2, *q*, $J_{4_2,3}$ 18, $J_{4_2,4_1}$ 2 Hz), 5.03 (H-4_1, *q*, $J_{3,4_1}$ 10.0, $J_{4_2,4_1}$ 2 Hz), 4.78 (H-1′, *d*, *J* 8 Hz), 4.50–3.50 (H-2′, 3′, 4′, 5′, and 2H-6′, *m*), 1.43 and 1.37 ($2CH_3$, 2*s*). MS: *m/e* 163(M^+-85). (Found: C 53.07; H 7.93. $C_{11}H_{20}O_6$ requires: C 53.21; H 8.12%).

Tetraacetyl-derivative (**2**). Ttreatment of compound **1** (100 mg) with Ac_2O–Py for 48 hr at room temp gave **2** (80 mg); mp 113–114° (Me_2CO/Hexane); $[\alpha]_D^{25°}$ −6° (*c* 1.08; $CHCl_3$); ν_{max}^{KBr} 1760, 1745, 1380, 1258, 1225 cm^{-1}; NMR ($CDCl_3$): δ 5.84 (H-3, *q*, $J_{3,4_1}$ 10 and $J_{3,4_2}$ 17.5 Hz), 4.53 (H-1′, *d*, $J_{1',2'}$ 8.0 Hz), 4.20 (H-$6'_1$, *q*, $J_{5',6'_1}$ 5.5, $J_{6'_1,6'_2}$ 11.5 Hz), 4.04 (H-$6'_2$, *q*, $J_{5',6'_2}$ 2.5, $J_{6'_1,6'_2}$ 11.5 Hz), 3.62 (H-5′, *m*), 2.06, 2.08, 2.00, 1.98 (4 OAc 4*s*), 1.33 and 1.27 (2Me, 2*s*). MS: *m/e* 347(M^+-63). (Found: C 54.65; H 6.97. $C_{19}H_{28}O_{10}$ requires: C 54.80; H 6.78%).

Dihydro-derivative (**3**). **1** (100 mg) in EtOH with 10% Pd–C (40 mg) at room temp, gave **3**; mp 120–121° (MeOH–Et_2O); $[\alpha]_D^{20°}$ − 31° 0.94; Py); ν_{max}^{nujol} 3460 cm^{-1}; NMR(Py): δ 4.84 (H-1′, *d*, $J_{1',2'}$ 8.0 Hz), 1.62 (2H, *q*, *J* 6 Hz, Me–CH_2–C–), 1.28 (6H, *s*, $C(Me)_2$), 0.95 (3H, *t*, –CH_2–CH_3). Found: C 53.35; H 8.86. $C_{11}H_{22}O_6$ requires: C 52.78; H 8.86).