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L-3,4-DIHYDROXYPHENYLALANINE-3-SULFATE FROM THE BROWN ALGA, *ASCOPHYLLUM NODOSUM*[1]

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During a study of free amino acids of marine macrophytic algae, an unidentified compound was detected in extracts of *Ascophyllum nodosum* (L.) Le Jolis. On an automatic amino acid analyzer, the unknown compound eluted almost at the front and was quantitatively the major component detectable with ninhydrin. High-voltage paper electrophoresis at pH 6.5 (pyridine-acetate buffer) caused the unidentified compound to migrate towards the anode, whereas at pH 1.8 (acetate-formate buffer) it lacked mobility, indicating a covalently bound, strongly acidic group. The acidic group responsible for this mobility pattern was completely lost within a few minutes in 6 N HCl at room temperature, and above pH 7 solutions δ turned brown at a similar rate. Consequently, care was required for successful extraction and purification. The unknown compound was not detected in extracts from *Polysiphonia lanosa* (L.) Tandy, a common epiphyte on *A. nodosum*.

The native compound was partially purified by adsorption onto charcoal and by filtration through a bed of cation exchange resin. Mild hydrolysis conditions were used to remove the strong acid moiety, then further purification was achieved by adsorption onto a cation exchange resin. After careful elution from the resin and concentration by evaporation, the hydrolyzed compound crystallized. After recrystallization, physical and chemical properties were consistent with those of L-DOPA. Further examination by ^{13}C-nmr spectroscopy (Table 1) confirmed the identification.

TABLE 1. ^{13}C-nmr of the Hydrolyzed Compound (L-DOPA)[a]

C-	shift, ppm	$^{1}J_{CH}$ (Hz)	mult.	$^{n}J_{CH}$ (Hz)	(n=)	mult.
1	127.9	——	s	6.5	3	d of d
2	118.7	156	d	5-5.5	3	d of t
3	145.9	——	s	3-3.5	2	d
				7	3	d
4	145.3	——	s	7.5	3	t
5	118.3	160	d	——	—	—
6	123.4	160-161	d	5-5.5	3	d of t
1′	36.4	132	t	3.5	2	d
				4	3	t
2′	55.7	147	d	3-4	2	t
3′	172.7	——	s	6	2	d
				4	3	t

[a]D_2O-H_2O 1:1 (v:v) 0.1 N in HCl, referenced to DMSO=40.48 ppm (1). Spectra were recorded at ambient temperatures on a Varian FT-80 spectrometer (20.11 MHz) using internal ^{2}H lock. For broadband-^{1}H-decoupled spectra, acquisition times were 0.991 s, flip angle 49°, and pulse delay 0 s. Corresponding parameters for high-resolution spectra were 1.982 s, 57° and 1.50 s respectively. Acidification shifts thge DMSO signal approximately 0.2 ppm downfield relative to sodium 3-trimethylsilylpropionate-2,2,3,3-^{2}H.

The strongly acidic group of the L-DOPA derivative precipitated with $BaCl_2$, and the presence of sulfur was confirmed by electron microprobe analysis. Sulfate was determined by weighing

[1]NRCC No. 23018.

the barium salt after hydrolysis of a fresh sample, purified by electrophoresis; 53.6 μmol $BaSO_4$ was recovered from 55 μmol of the compound, indicating only one sulfate per molecule. The position of the sulfate ester was established by ^{13}C-nmr of the unhydrolyzed compound. Experimentally determined chemical shifts (Table 2) were compared with expected values calculated using substituent effects described earlier (2) for phenyl sulfates, and the difference (summed over all aromatic-ring signals) was used as an indicator of agreement (3). Values determined for this index [Σ (observed-expected)] were: DOPA-3-SO_4, 5.61 ppm; DOPA-3,6-diSO_4, 19.67 ppm; DOPA, 23.77 ppm; and DOPA-4-SO_4, 42.87 ppm. Thus, the unknown amino acid was identified as L-DOPA-3-SO_4. This novel natural product is the major free amino compound for most of the year. It is evenly distributed in different parts of the plant, and concentrations range seasonally between 0.1% (winter) and 0.05% (summer) of the fresh weight.

TABLE 2. ^{13}C-nmr of the Native Compound (L-DOPA-3-SO_4)[a]

C-	shift, ppm	$^1J_{CH}$ (Hz)	mult.	$^nJ_{CH}$ (Hz)	(n=)	mult.
1	125.9	——	s	n/a[b]	—	n/a
2	124.3	160	d	4-5	3	d of t
3	140.8	——	s	ca. 5	2	d
				ca. 5	3	d
4	148.7	——	s	6.4	3	t
5	117.4	160	d	——	—	——
6	126.2	ca. 159	d	4-5	3	d of t
1′	35.2	ca. 131	t	n/a	—	n/a
2′	53.7	146	d	n/a	—	n/a
3′	171.9	——	s	n/a	—	n/a

[a]Solvent, $^2H_6$-DMSO. Reference, central line of $^2H_6$-DMSO=39.56 ppm (2). See footnote to Table 1 for instrument parameters and reference shift.

[b]n/a=data not available from spectra due to incomplete resolution.

Although well known as an intermediate in the biosynthesis of melanin and catecholamines, L-DOPA has been reported to accumulate in only a few higher plants (4). We were unable to detect either L-DOPA or its sulfate in several other common local seaweeds. *A. nodosum* contains large quantities of polyphenols (5), including at least one disulfate, 1,2,3,5-tetrahydroxybenzene 2,5-disulfate (3). Sulfation of L-DOPA, however, appears to be specific to the 3 position only, as we found no evidence for the 4-sulfate or the disulfate esters. Phenol sulfates have long been known as excretion products in animals (6). Interestingly, dopamine-3-SO_4 and to a lesser extent, dopamine-4-SO_4, are excreted by patients with Parkinson's disease during treatment with L-DOPA (7). In *A. nodosum*, L-DOPA-3-SO_4 may simply be a stabilized by-product. On the other hand, there could be a distinct physiological role as in certain legume seeds where, in high concentrations, L-DOPA appears to act as a feeding repellent (8).

EXPERIMENTAL

ISOLATION PROCEDURES.—*L-DOPA-3-SO*$_4$. Extractions were made from 5 kg samples of fresh or frozen *A. nodosum* collected at Morris Point, Halifax Co., Nova Scotia. Whole plants were soaked for two to three days in 10 liters of 1 mM HCl, releasing about 3 g of the compound, determined by amino acid analysis of an aliquot. The extract was decanted, and 200 g of Norit A charcoal powder was stirred in, the charcoal was subsequently collected by filtration, washed with 1 liter of H_2O, resuspended in 500 ml of 50% aqueous EtOH, and finally adjusted to pH 5.5 with NaOH. The charcoal was removed by filtration, and the filtrate concentrated to about 100 ml by rotary evaporation. This filtrate was passed through a column of Dowex 50×8 H^+ (7 cm diameter×16 cm), and the column washed with

H_2O. Uncharged and other acidic low molecular weight compounds were eluted in the first 450 ml and the unidentified compound was collected in the next 800 ml. Mannitol was the major contaminant and was removed by paper electrophoresis at pH 3.5 (pyridine-acetate buffer, Whatman 3MM paper 46 cm width) but this gave a pyridinium salt. Paper electrophoresis at pH 1.8 (8% HOAc, 2% HCO_2H) gave the free acid.

L-DOPA. HCl (25 ml) was added to the 800 ml eluate of the Dowex-50 column, and the solution was concentrated on a rotary evaporator at 40°, during which the compound was quantitatively hydrolyzed. These conditions are known to hydrolyze phenyl sulfate esters (1). Dowex 50×8 H^+ (50 g) was then added, filtered, and washed with H_2O, and a suspension in 200 ml of H_2O was slowly titrated to pH 5.5 with NaOH solution and again filtered. A white, crystalline material precipitated during evaporation to 10 ml. This material was washed three times with small volumes of cold H_2O, dissolved in hot 50% aqueous HOAc, and recrystallized on adding *n*-BuOH. The hydrolyzed compound eluted from the amino acid analysis column with the pH 4.25 buffer immediately following leucine and at the same position as 3,4-dihydroxyphenylalanine (DOPA). The yield was ~300 mg, or about 10% overall recovery from the initial extract, mp 275° (dec.) (lit. 276-278°), $[\alpha]^{20}D$ −10.5° (c=2.0 in N HCl) (lit. −13.1°, c=5.12 in N HCl). Found: C 53.85, H 5.76, O 33.33, N 7.06%. Required for L-DOPA, C 54.82, H 5.62, O 32.46, N 7.10%. The ir spectrum in Nujol was identical with that of L-DOPA. The identity of the hydrolyzed compound as L-DOPA was confirmed by ^{13}C-nmr (Table 1).

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