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CHARACTERIZATION OF DOPA BETAINE, TYROSINE BETAINE AND *N*-DIMETHYLTYROSINE FROM *LOBARIA LAETEVIRENS*

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Abstract—DOPA betaine, tyrosine betaine and *N*-dimethyltyrosine were isolated from the lichen *Lobaria laetevirens*. Their structures were determined by radiochemical and spectroscopic methods and by comparisons with synthetic samples. Their low level in the thallus and their high specific radioactivity suggest that they might be active metabolites.

INTRODUCTION

During studies on the *N*-methylated compounds from *Lobaria laetevirens* (Lightf.) Zahlbr. and after the characterization of sticticin, the methyl ester of DOPA betaine [1], a new compound which also gives a positive response with Dragendorff reagent, was found (**1**). Although this compound was far less abundant than sticticin, a sufficient amount was isolated for spectroscopic analysis. Furthermore, during experiments with the radioactive compounds, L-[methyl-^{14}C]-methionine and L-[U^{14}C]tyrosine, two other compounds (**2** and **3**) were also found to be highly labelled and *N*-methylated. In the present paper we describe the structure of these three new compounds.

RESULTS AND DISCUSSION

L. laetevirens contains low levels of **1**; thus an extract from 25 g dry plant material only produced 20 mg of **1** as the hydrochloride. A ^{1}H NMR spectrum was obtained from a solution of the hydrochloride in D_2O, at 100 MHz: it gave the following signals (ppm/TMPS): 3.15 (*m*, 2H $\underline{CH_2}$-Ar); 3.27 (*s*, 9H, $N^+\underline{Me_3}$); 3.81 (*m*, 1H, $\underline{CH}$–CH_2); 6.48 (*m*, 3H, Ar). This spectrum exhibits characteristics similar to those shown by DOPA betaine, which was produced by a 10.5 N HCl treatment of sticticin at 150° [1]. Moreover, it seems identical to that of *N*-trimethyl DOPA synthesized in this laboratory. A coelectrophore-chromatography run with **1**, isolated from the thallus, and the synthetic betaine showed only one spot with the Dragendorff reagent. All these results show **1** is DOPA betaine.

During experiment using L-[U^{14}C]tyrosine, two compounds, **2** and **3**, appeared highly radioactive. Since they could not be detected on chromatograms by either ninhydrin or Dragendorff reagent, their isolation for spectroscopic studies was not possible. As a result of the high apparent specific radioactivity of **2**, the determination of its structure was investigated by chemical analysis. An aliquot of **2** treated with N NaOH, at 80° during 30 min, gave radioactive *p*-coumaric acid. Consequently, **2** contains the carbon skeleton of tyrosine. In view of the rapid loss of the amino group, it appears that the amino-*N* is substituted with methyl groups. In order to verify this hypothesis L-[methyl-^{14}C]methionine was fed to the thallus. On alkaline distillation (2 N NaOH at 80°), radioactive **2** produced a volatile base which was identified as trimethylamine by coelectrophoresis. Thus **2** appears to be *N*-trimethyltyrosine, i.e. tyrosine betaine. Proof of this was provided by synthesizing tyrosine betaine. The main product present in the reaction mixture was isolated; its NMR spectrum carried out in D_2O at 100 MHz gave the following data (ppm/TMPS): 2.85 (*m*, 2H, $-\underline{CH_2}$–Ar); 3.02 (*s*, 9H, N^+Me_3); 3.87 (*m*, 1H, $\underline{CH}$–CH_2–); 6.77 (*m*, 4H, Ar), which corresponded to those of tyrosine betaine. Isolated **2**, coelectrophore-chromatographed with the synthetic tyrosine betaine showed strict coincidence after autoradiography and revelation with Dragendorff reagent.

Compound **3** appeared strongly labelled after incorporation of either L-methionine or L-tyrosine. It might therefore by a byproduct of tyrosine metabolism, for example an *N*-methylderivative. A coelectrophore-chromatography carried out with an EtOH-soluble fraction from the L-[U^{14}C]tyrosine experiment contained **3**, and a synthetic sample of *N*-dimethyl-tyrosine showed a perfect superimposition of the radioactive spot of **3**. Thus **3** was identified as *N*-dimethyltyrosine.

Of the three *N*-methylated compounds isolated from *L. laetevirens*, DOPA betaine is by far the most important; however, its level does not exceed 10 μmol/g dry wt. It has been detected in all species which have sticticin [1]. Tyrosine betaine and *N*-dimethyltyrosine are also present at very low level; since their specific radioactivities were relatively high during labelling experiments, they appear to be intermediates in the biosynthesis of sticticin from tyrosine.

EXPERIMENTAL

Extraction and purification of DOPA betaine. Lyophilized pieces of thallus were powdered in a Dangoumeau's grinder. The resulting powder (5 g) was extracted at 4° with 50 ml

MeOH–$CHCl_3$–H_2O (12:5:3). After centrifugation and filtration, 25 ml $CHCl_3$ and 25 ml H_2O were added to the extract and the preparation kept at 4° overnight. The upper phase, collected after decantation was evapd to dryness at 30°. The residue was dissolved in H_2O. DOPA betaine was isolated and purified by high voltage paper electrophoresis and PC using the systems previously described for sticticin [1].

Tyrosine betaine and N-*dimethyltyrosine.* These two compounds occurred at very low levels in plant material. Since they appeared highly radioactive in experiments using radioactive L-tyrosine and L-methionine, they were labelled *in situ* at the highest specific radioactivity by means of these two amino acids. 50 μCi of L-[U^{14}C]tyrosine and 25 μCi of L-[methyl-^{14}C]methionine, purchased from the CEA (France) were applied (1 ml each) to 350 mg thallus for 24 hr. Thalli were then dipped into liquid N_2 (4 min) and then ground in a Dangoumeau's grinder. Powder was taken up by 15 ml EtOH acidified with HCl (EtOH 80°, HCl 0.01 N) and extrd for 15 min at low temp. After centrifugation and filtration the extract was evapd to dryness at 30° and the residue dissolved in H_2O. An aliquot of this solution was chromatographed on paper in *n*-BuOH–HOAc–H_2O (12:3:5). The chromatogram was dried at 40° and then submitted to autoradiography for 10 days. Radioactive compounds were eluted from the paper and purified further by high voltage electrophoresis with 3% HCOOH (pH 2.0) as solvent.

After elution and evapn, purity was checked for each compound by autoradiography. Two distinct radioactive tyrosine betaines were obtained; one was labelled on the ring and the side-chain (from L-[U-^{14}C]tyrosine), the other on the methyl groups (from L-[methyl-^{14}C]methionine). *N*-Dimethyltyrosine also appeared to be radioactive but its isolation without radioactive contamination was not possible. However, it was easily located on chromatograms and comparison was made with an authentic synthetic sample.

Electrophoresis and chromatography. High voltage paper electrophoresis and PC were used. The electrophoretic migration (EM) in comparison with that of choline [2] was determined on Whatman 3 MM paper at 40 V/cm and pH 2.0 (HCOOH 3%). EM was 0.27 for the two betaines, 0.39 for *N*-dimethyltyrosine. R_f values on Whatman 3 MM paper in *n*-BuOH–HOAc–H_2O (12:3:5) were for DOPA betaine 0.36, for tyrosine betaine and *N*-dimethyltyrosine 0.50. When the solvent PhOH–EtOH–H_2O (15:4:1) was used, the R_fs were respectively 0.84, 0.91 and 0.78. Trimethylamine as the hydrochloride was characterized by high voltage electrophoresis (40 V/cm) using Whatman 3 MM paper and 6% HCOOH. *p*-Coumaric acid was identified by 2-D PC on Whatman 3 MM paper using *n*–BuOH–HOAc–H_2O (12:3:5) and 2% HOAc. The compounds were detected on paper by spraying Dragendorff reagent for betaines and trimethylamine, ninhydrin reagent for *N*-dimethyltyrosine; UV detection at 254 and 350 nm was used for *p*-coumaric acid and also for the three first compounds cited. Finally, radioactive compounds were autoradiographed with Kodirex films.

Radiochemical counting procedure. Radioactive liquid samples spotted on Whatman paper, and radioactive areas of autoradiograms were burnt into the Packard Sample Oxidizer model 306. CO_2 collected as carbamate in Carbosorb was dissolved in scintillation mixture (Permafluor). The radioactive samples were counted in a liquid scintillation spectrometer using an external standard.

DOPA betaine synthesis. L-DOPA (500 mg) was dissolved in 5 ml aq. $NaHCO_3$ (210 mg) in N_2 at 20°. Me_2SO_4 (195 mg) was added with stirring. After 24 hr the pH became neutral and water was evapd; the residue was suspended in MeOH and filtered. Then Na_2SO_4 and the remaining Me_2SO_4 were discarded. After MeOH had been removed, 1 g of a solid mixture containing DOPA betaine but also its methyl ester (sticticin) and small amounts of the corresponding methoxylated compounds were collected. DOPA betaine and its methyl ester were isolated from the reaction and purified by PC and high voltage paper electrophoresis.

Tyrosine betaine synthesis. Synthesis of tyrosine betaine was carried out according to the method of Corti [3] modified for the hydrolysis step which was made here with conc HCl for 40 hr. The hydrochloride had mp 207°.

N-*Dimethyltyrosine synthesis.* A reaction was initiated between dimethylamine and 2-bromo-3-(*p*-hydroxyphenyl)propanoic acid [4].

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