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N-BENZOYLPHENYLALANINE AND *N*-BENZOYLPHENYLALANINOL, AND THEIR BIOSYNTHESIS IN *PENICILLIUM BREVICOMPACTUM*

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Abstract—*N*-Benzoylphenylalanine and *N*-benzoylphenylalaninol have been detected in cultures of *Penicillium brevicompactum*. Isotope from [U-^{14}C]phenylalanine was incorporated effectively into the benzoyl group of both compounds.

INTRODUCTION

During a RGC study of how the production of mycophenolic acid and a series of simpler acetogenic phenols (the 'Raistrick' phenols) correlated with the development of *Penicillium brevicompactum* in liquid culture [1], two new major metabolites of the fungus were encountered. They appeared as two sizeable peaks in chromatograms derived from methylated total culture extracts obtained after 76 hr of the fermentation. Isotope from [^{14}C]acetate was not transferred in a 2-hr incubation into either of the new metabolites. Fragment ions characteristic of the benzoyl unit were prominent in the mass spectrum of both materials. Our initial belief was that these metabolites were related to the pebrolides, a set of sequiterpenoid benzoates that have previously been isolated from the fungus [2, 3]. This is not the case; the materials are the *N*-benzoyl derivatives of phenylalanine and phenylalaninol. The ester of these two materials, asperphenamate [4, 5], has also been detected in *P. brevicompactum*.

RESULTS AND DISCUSSION

The two new metabolites, as components of a total culture extract that had been methylated with CH_2N_2 prior to analysis, eluted from 3% OV-17 gas chromatographic columns between the *n*-C_{24} and *n*-C_{28} hydrocarbons. (The metabolites are responsible for the two large peaks following component G in the 76 hr mass panel in Fig. 4 of ref. [1].) Both substances had prominent *m/z* 105 ions in their mass spectra together with the *m/z* 77 fragment ion and the 56.5 amu metastable ion characteristic of the troponium cation, $C_6H_5CO^+$. In addition, the more abundant of the two metabolites had a M^+ at *m/z* 255 and major fragment ions at *m/z* 224 and 164. These data were in accord with the metabolite being *N*-benzoylphenylalaninol; a material that previously has been isolated from *Aspergillus flavipes* [4], *Catharanthus pusillus* [6] and *Alangium lamarckii* [7]. This assignment was confirmed by comparing the R_f (two columns, one capillary) of the isolate with a sample prepared from benzoyl chloride and L-phenylalaninol by the method of Battersby and Kapil [6], and with an authentic sample kindly provided by Dr. N. J. McCorkindale [5].

The second metabolite had a M^+ of *m/z* 283 and showed major fragment ions at *m/z* 224 and 162. These data indicated that the metabolite might be the Me ester of *N*-benzoylphenylalanine, and this was confirmed by R_f and mass spectral comparison with synthetic and authentic samples (the latter again kindly provided by Dr. N. J. McCorkindale [5]).

Finding the *N*-benzoyl derivatives of both phenylalanine and phenylalaninol in *P. brevicompactum* immediately suggested that their ester, asperphenamate, might also be present. Preliminary GC and HPLC data support this suggestion.

Unsubstituted benzoyl units are uncommon in natural products; indeed *P. brevicompactum* appears to be one of the most prolific producers of them. Biosynthetically the origin of this unit is interesting. The shikimic acid pathway is the most likely source of the carbon but it is not immediately obvious whether or not phenylalanine is a necessary intermediate. To explore this question, a liquid surface culture that was 68 hr old was treated with [U-^{14}C]-L-phenylalanine for 24.5 hr. An isotope dilution analysis was performed with unlabelled *N*-benzoylphenylalanine and the corresponding benzoyl alcohol. The incorporation value for the conversion of [U-^{14}C]-L-phenylalanine to *N*-benzoylphenylalanine and *N*-benzoylphenylalaninol were 0.06 and 0.43%, respectively.

The benzoyl group was then removed from both the *N*-benzoylphenylalanine and the *N*-benzoylphenylalaninol by the procedure of White [8, 9]. Following rigorous purification of the benzoic acid produced, it was found that the benzoyl group associated with phenylalaninol contained 44.7% of the total radioactivity of the parent amide while the benzoyl group associated with phenylalanine contained 40.3% of the total radioactivity of the other parent amide. These data establish that both benzoyl groups derive from phenylalanine.

The incorporation and degradation data contain one other significant piece of information. Although the incorporation of precursor activity into the two amides differed by a factor of 7, the proportion of total

incorporated activity that was present in each benzoyl group was essentially the same. Moreover, that proportion was very close to the theoretical value of $7/16 = 43.8\%$ which would result if all the carbon required to construct the carbon skeleton of either of the amides was removed from the precursor pool at the same point in time. The implications of this finding are being studied.

EXPERIMENTAL

Mps are uncorr. NMR were recorded at 60 MHz using TMS as int. standard.

Syntheses. *N*-Benzoylphenylalanine was prepared from DL-phenylalanine and benzoyl chloride according to ref. [10]. The product crystallized from HOAc–C_6H_6 as needles, mp 185–186°, lit. [10] 188°. MS 70 eV *m/z* (rel. int.): 269 (M^+, 3), 225 (6), 148 (53), 147 (21), 122 (6), 105 (100), 91 (43), 77 (93). The Me ester (CH_2N_2) crystallized from C_6H_6–hexane as needles, mp 85–87°, lit. [4] 87°. MS 70 eV *m/z* (rel. int.): 283 (M^+, 1), 224 (5), 162 (45), 161 (8), 105 (100), 91 (23), 77 (55). ^{1}H NMR ($CDCl_3$): δ 3.2 (*d*, $C\underline{H}_2$), 3.7 (*s*, $OC\underline{H}_3$), 5.1 (*m*, $C\underline{H}$), 7.0–7.8 (*m*, 10 aromatic $\underline{H}$).

N-Benzoylphenylalaninol was prepared from L-phenylalaninol and benzoyl chloride according to the method of ref. [5]. The product crystallized from EtOH–CCl_4 as needles, mp 171–173°, mmp with authentic sample [5] 171–173°. MS 70 eV *m/z* (rel. int.): 225 (M^+, 1), 224 (4), 164 (31), 122 (6), 105 (100), 91 (27), 77 (68).

Biosyntheses. [U-^{14}C]L-phenylalanine (90 μCi, 464 μCi/μmol) was fed to a 68-hr-old liquid surface culture of *P. brevicompactum* (ATCC 9056) established in Czapek–Dox medium (100 ml) in a conical flask (250 ml). Following a 24.5-hr incubation, the culture was homogenized and samples of unlabelled *N*-benzoylphenylalanine (406.4 mg) and *N*-benzoylphenylalaninol (407.1 mg) were added to a portion (21.5 ml) of the total homogenate (88 ml). These latter *N*-benzoyl derivates were then re-isolated from the homogenate and recrystallized to constant sp. act. The final sp. act. were 507 and 71 dpm/mg for the alcohol and acid, respectively, yielding incorporation values of 0.43 and 0.06%, respectively, for the alcohol and acid.

The benzoyl moieties of both *N*-benzoylphenylalanine and *N*-benzoylphenylalaninol were removed from the parent molecule by first converting them to their *N*-nitrosamides with $NaNO_2$ in Ac_2O [8] then decomposing the nitrosamides thermally [9]. The recovered benzoic acid samples were crystallized to constant sp. act., 474 and 63.4 dpm/mg for that derived from the alcohol and acid, respectively.

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