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N-CORONAFACOYL-L-ISOLEUCINE AND *N*-CORONAFACOYL-L-ALLOISOLEUCINE, POTENTIAL BIOSYNTHETIC INTERMEDIATES OF THE PHYTOTOXIN CORONATINE

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Key Word Index—*Pseudomonas syringae* pv. *glycinea*; bacterium; toxin; leaf chlorosis; coronatine; *N*-coronafacoyl-L-isoleucine; *N*-coronafacoyl-L-alloisoleucine; biosynthesis.

Abstract—Two new naturally-occurring analogues of the phytotoxin coronatine have been isolated from liquid cultures of *Pseudomonas syringae* pv. *glycinea*. These have been identified as *N*-coronafacoyl-L-isoleucine and *N*-coronafacoyl-L-alloisoleucine by mass spectrometry and by studies of the products of acid hydrolysis of the two compounds. The compounds were purified as a mixture of *ca* 2:1 composition, but the two parent components were not preparatively separated. The possible significance of the two compounds, to the biosynthesis of coronatine, is discussed.

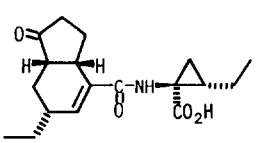
INTRODUCTION

Coronatine, a phytotoxin that induces chlorosis in young green leaves, is the major phytotoxic component produced in liquid culture by *Pseudomonas syringae* pv. *atropurpurea* (Reddy & Godkin 1923) Young, Dye & Wilkie 1978, and *Pseudomonas syringae* pv. *glycinea* (Coerper 1919) Young, Dye & Wilkie 1978 [1–3]. Liquid cultures of some strains of each of these two organisms are known to contain various components that are chemically, and probably biosynthetically, related to coronatine [4]. These are coronafacic acid [1, 5], *N*-coronafacoyl-L-valine [5] and norcoronatine [6].

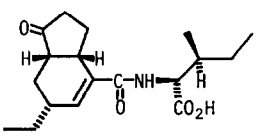
During the course of the isolation of norcoronatine from an extract of *P. s. glycinea*, the occurrence of a nonhomogeneous fraction with chlorosis-inducing activity was observed [6]. Data are now presented that characterize the two components found to constitute this fraction.

RESULTS AND DISCUSSION

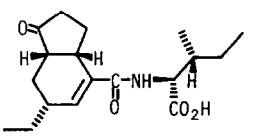
When the carboxylic acid fraction extracted from the culture medium of *P. s. glycinea* 4182 was chromatographed on LH20 Sephadex, as described in [6], a biologically active fraction was obtained that had a slightly longer retention time than *N*-coronafacoyl-L-valine and the major component, coronatine. This was purified by CC (silica gel) and prep. TLC. Although the preparation was apparently homogeneous on TLC, examination of the methyl ester by GC revealed a peak that had an unresolved shoulder indicating nonhomogeneity. Capillary GC resolved the mixture and demonstrated the presence of two components in a ratio of *ca* 2:1. The mass spectrum (GC-MS) of each component displayed an $[M]^+$ at *m/z* 335 and a base peak at *m/z* 191 was indicative of a *N*-coronafacoyl-substituted amino acid [5]. Although the mass spectra of the two compounds were almost identical, there was a consistent difference in the



coronatine



1 N-coronafacoyl-L-isoleucine



2 N-coronafacoyl-L-alloisoleucine

relative intensity of a fragment ion at *m/z* 279. Assuming the derivatization produced a monomethyl ester, these data correspond to stereoisomers of *N*-coronafacoylisoleucine for each component, rather than the isomeric *N*-coronafacoylleucine, the ion at *m/z* 279 arising from the loss of but-1-ene from the $[M]^+$.

A study of the products from an acid hydrolysis of the preparation gave results consistent with the above interpretation. An ethyl acetate extractable acidic compound was isolated from the hydrolysate and this was identified by GC and mass spectrometry as coronafacic acid [1, 5]. The water-soluble portion of the hydrolysis product was examined by 2D-thin layer electrophoresis (TLE)/TLC. The plates were visualized after ninhydrin treatment and spot positions were compared with those of high sp. act. [U-^{14}C]isoleucine and [U-^{14}C]leucine co-spotted as reference markers at the origin with the hydrolysis product. Isoleucine was a major component of the hydrolysate, but leucine was not detected. There was however, ninhydrin colour reactivity that extended beyond that attributable to isoleucine, but this second component was incompletely resolved from isoleucine. It travelled in the same position, relative to isoleucine, as alloisoleucine.* Conclusive identification of the amino acid products was obtained by GC of their methyl ester/*N*-trifluoroacetyl derivatives, compared with standards prepared from D- and L-stereoisomers of isoleucine, alloisoleucine and leucine. Two major components from the hydrolysis were present corresponding to L-isoleucine and L-alloisoleucine in a ratio of *ca* 2:1. Accordingly, the two parent compounds are *N*-coronafacoyl-L-isoleucine (**1**) (the more abundant) and *N*-coronafacoyl-L-alloisoleucine (**2**), where the coronafacoyl moiety has been arbitrarily assigned the *cis* configuration, as determined previously for the parent compound coronatine [1], and proposed for other analogues of coronatine [5, 6]. One of these compounds **1**, has previously been synthesized and chemically characterized [7]. However, to our knowledge this is the first report of **1** and **2** as metabolic products.

The natural occurrence of compounds **1** and **2** is of considerable interest from a biosynthetic viewpoint, because either or both of these two compounds may be intermediates in the biosynthesis of coronatine. Although this has been suggested to involve coupling of L-isoleucine and coronafacic acid to form *N*-coronafacoyl-L-isoleucine [8], nothing is yet known about the stereochemical requirements in the cyclization step(s) leading to the cyclopropyl ring of coronatine. In this respect it will be of interest to determine whether either or both of compounds **1** and **2** lie on the biosynthetic pathway to coronatine.

*The three compounds isoleucine, alloisoleucine and leucine were only partially resolved when run together on 2D TLE/TLC. Leucine and isoleucine had the same electrophoretic mobility that was greater than that of alloisoleucine. Chromatographically, isoleucine and alloisoleucine moved together, but were slower than leucine.

EXPERIMENTAL

P.s. glycinea 4182 was from the Plant Diseases Division Culture Collection. Procedures of culture and general chemical procedures are described elsewhere [5]. CC on LH20 Sephadex and the derivatization of amino acids and their GC is described in [6]. The TLC dimension of 2D TLE/TLC adopted a double elution, first with MeCOEt–pyridine–H_2O–HOAc (70:15:15:2), then with *n*-PrOH–H_2O–*n*-PrOAc–HOAc–pyridine (120:60:20:4:1) as described in ref. [9].

Capillary GC (for GC-MS) was on a 0.3 mm ID × 25 m OV101 fused silica WCOT column, with He at 25 cm/sec. The sample was applied to stainless steel traps packed with 5% SE30 on Chromsorb W-HP [10] and inserted into the injector held at 175°. The oven temp. was prog from 130 to 216° at 10°/min.

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