Phytochemistry, Vol. 26, No. 10, pp. 2695–2697, 1987.
Printed in Great Britain.

0031–9422/87 $3.00 + 0.00
Pergamon Journals Ltd.

ABSOLUTE CONFIGURATION OF NORCORONATINE

ROBIN E. MITCHELL, MICHAEL C. PIRRUNG* and GERARD M. MCGEEHAN*

Division of Horticulture and Processing, DSIR, Private Bag, Auckland, New Zealand; *Department of Chemistry, Stanford University, Stanford, California 94305, U.S.A.

(*Received* 24 *September* 1986)

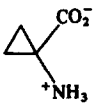
Key Word Index—Aminocyclopropane carboxylic acid; coronamic acid; coronatine; phytotoxin; norcoronamic acid; norcoronatine; *Pseudomonas syringae* pv *glycinea*; absolute stereochemistry; biosynthesis.

Abstract—Norcoronamic acid was established as having the absolute stereochemistry (1*S*,2*S*)-2-methyl-1-aminocyclopropane-1-carboxylic acid by comparison of its properties with synthetic preparations of the (1*R*,2*R*), (1*R*,2*S*) and (1*S*,2*R*) stereoisomers.

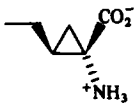
INTRODUCTION

A number of compounds of the aminocyclopropane carboxylic acid class have been isolated and identified in recent years. The first of these, 1-aminocyclopropane-1-carboxylic acid (1 ACC), has been known for some time as a plant product [1] and has been shown to be the precursor to the ripening hormone, ethylene, in higher plants [2]. Coronamic acid (2) is found as an amide derivative in the phytotoxin coronatine [3]. The structure of coronamic acid has been confirmed by synthesis [4] and its absolute configuration has been established [5].

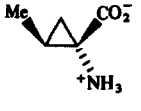
A recent report [6] has shown norcoronamic acid (3) to be a structural component of norcoronatine, a minor component of the phytotoxic fraction of *Pseudomonas syringae* pv. *glycinea*. However, it was only possible to provide a tentative proposal of the stereochemistry of the norcoronamic acid moiety of norcoronatine, based on analogy to coronatine and the probable parallel biosynthetic route to the two analogues. This report presents proof of the relative and absolute configuration of norcoronamic acid and thus of norcoronatine, and discusses the implications this has for the biosynthesis of this class of compound.



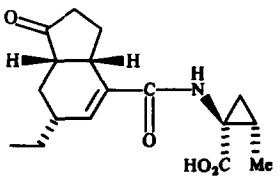
1



2



3



4

RESULTS AND DISCUSSION

The properties of 2-methyl-1-aminocyclopropane-1-carboxylic acid (norcoronamic acid) obtained from the acid hydrolysis of norcoronatine were compared with the properties of synthetic stereoisomers of 2-methyl-1-aminocyclopropane-1-carboxylic acid: (1*R*,2*S*) and its (1*S*,2*R*) enantiomer, and the (1*R*,2*R*) diastereoisomer.

The ^{1}H NMR spectra of our synthetic preparations of the (1*S*,2*R*) and (1*R*,2*R*) diastereoisomers were in close agreement with the data reported [7] for racemic mixtures of each of these diastereoisomers (Table 1). The ^{1}H NMR spectrum of the hydrochloride salt of norcoronamic acid, obtained from the acid hydrolysis of norcoronatine, was the same as that of the hydrochloride salt of the (1*R*,2*R*)-stereoisomer (Table 1), and therefore establishes the relative configuration of norcoronamic acid as in (1*R*,2*R*) and (1*S*,2*S*) enantiomers. This finding was consistent with the results of 2D TLE/TLC of the three synthetic stereoisomers and norcoronamic acid. Relative to the three reference compounds ACC, ^{14}C-val and ^{14}C-ile, the (1*R*,2*R*) stereoisomer migrated to a different position than did the (1*S*,2*R*) stereoisomer (which had the same position as its enantiomer). The difference between the two spot positions was one spot diameter, mainly as a result of differing electrophoretic mobility, the (1*R*,2*R*) stereoisomer being the faster-moving. The position of norcoronamic acid on 2D TLE/TLC relative to the three reference compounds matched that of the (1*R*,2*R*) stereoisomer and was different from that of the (1*S*,2*R*) and (1*R*,2*S*) stereoisomers.

The absolute configuration of norcoronamic acid was determined from the different retention times on GC of derivatives of the stereoisomers using a chiral liquid phase [8] (Table 2). The retention time of norcoronamic acid was clearly different from that of the (1*R*,2*R*) stereoisomer and accordingly the configuration of norcoronamic acid must be the enantiomeric (1*S*,2*S*). The paucity of norcoronatine, and more so of norcoronamic acid, did not enable the optical rotation of norcoronamic acid to be determined for a comparison to be made with the other stereoisomers (Table 2). However, the data we have obtained conclusively shows that the absolute configuration of norcoronamic acid is as depicted in 3, and of

Table 1. Determination of the relative configuration of norcoronamic acid

Configuration	^{1}H NMR data (δppm) for proton on carbon no. 2-Me	3	2
1*R*, 2*R**	0.965(3H, *d*, *J*=6 Hz)	1.00–1.11(2H, *m*)	1.21–1.37(1H, *m*)
1*S*, 2*R*†	0.99(3H, *d*, *J*=6.4 Hz)	0.68(1 H, *dd*, *J* = 6, 7.5 Hz) 1.24(1H, *dd*, *J*=6, 9.5 Hz)	1.38–1.53(1H, *m*)
Norcoronamic acid. HCl	1.04(3H, *d*, *J*=6 Hz)	1.17–1.36(2H, *m*)	1.44–1.64(1H, *m*)
1*R*, 2*R*. HCl	1.03 (3H, *d*, *J*=6 Hz)	1.15–1.33(2H, *m*)	1.41–1.58(1H, *m*)
1*S*, 2*R*. HCl	1.03(3H, *d*, *J*=6.3 Hz)	0.89(1H, *dd*, *J*=6, 7.5 Hz) 1.45(1H, *dd*, *J*=6, 9.5 Hz)	1.55–1.73(1H, *m*)

^{1}H NMR data were compared for the hydrochloride salts of norcoronamic acid and (1*R*,2*R*) and (1*S*,2*R*)-2-methyl-1-aminocyclopropane-1-carboxylic acid with published data. All spectra were recorded in D_2O and were referenced to dioxane at δ3.53 ppm as internal reference.

*As reported [7] for 1*R*, 2*R*/1*S*, 2*S* racemate.

†As reported [7] for 1*R*, 2*S*/1*S*, 2*R* racemate.

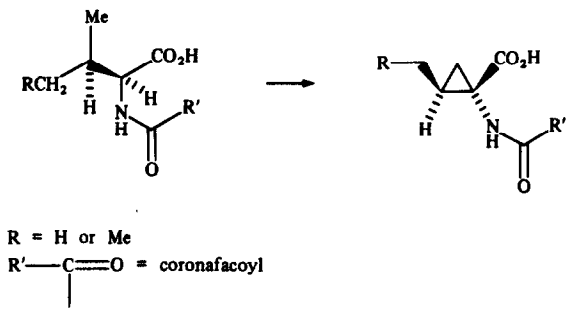
Table 2. Determination of the absolute configuration of norcoronamic acid

Configuration	R_t (min)	Opt. rot. $[\alpha]_D^{25}$ (°)
1*R*, 2*R*	7.35	−39
Norcoronamic acid	8.16	not determined
1*R*, 2*S*	8.55	+74.5
1*S*, 2*R*	8.85	−68.2

GC retention times were measured for methyl ester/*N*-trifluoroacetyl derivatives of synthetic stereoisomers of 2-methyl-1-aminocyclopropane-1-carboxylic acid and compared with that for norcoronamic acid.

norcoronatine as depicted in **4**, corresponding to that previously proposed [6].

The absolute stereochemistry of **3** as established herein as well as the isolation from liquid cultures of the coronafacoyl-derivatives of **3**, isoleucine [9] and valine [6] that are each amidated with the same carboxylic acid (coronafacic acid) that is found in coronatine, suggest a biosynthetic pathway for these compounds as shown in Scheme 1. The crucial step would be a 1,3-dehydrogenation which occurs with retention of configuration on the α-carbon and operates on the pro*S*-alkyl substituent of the amino acid amide. This corresponds to the methyl group of the isoleucyl amide. In accordance with this pathway, there may be a stereochemical imperative for enzymes involved in such transformations. The occurrence of these cyclopropane materials also suggests that other cyclopropane amino acid analogues may be found in Nature. In fact, a cyclopropyl arginine analogue has recently been isolated from a marine red algae *Grateloupia carnosa* [10].



Scheme 1.

EXPERIMENTAL

(1*R*,2*S*)-2-Methyl-1-aminocyclopropane-1-carboxylic acid and its (1*S*,2*R*) enantiomer were available as previously described [11]. (1*R*,2*R*)-2-Methyl-1-aminocyclopropane-1-carboxylic acid was obtained by applying the synthesis of ref. [7] to 1,2-dibromopropane prepared by our procedure [11]. It showed optical rotation of −39° and spectral data consistent with that of refs [7, 12]. Norcoronamic acid was obtained by the acid hydrolysis of norcoronatine [6]. For GC, norcoronamic acid and stereoisomers (1*S*,2*R*-; 1*R*,2*S*-; 1*R*,2*R*-) of 2-methyl-1-aminocyclopropane-1-carboxylic acid were first derivatized to their methyl ester/*N*-trifluoroacetyl derivatives. GC was as previously described [6]. 2D TLE/TLC was on cellulose MN300; the TLE dimension was at pH 2, and TLC was done sequentially with the following two solvent systems: butan-2-one–C_5H_5N–H_2O–HOAc (70:15:15:2) and propan-1-ol–H_2O–propan-1-yl acetate–HOAc–C_5H_5N (120:60:20:4:1). For reference markers, 1.5 μg ACC, and ^{14}C-valine and ^{14}C-isoleucine (sp. act. > 250 μCi/μmol) were co-spotted at the origin with the test sample. ^{1}H NMR spectra of stereoisomers of 2-methyl-1-aminocyclopropane-1-carboxylic acid and of norcoronamic acid were recorded at 200 MHz in D_2O with 0.5 μl dioxane added as int. ref.

REFERENCES

1. Burroughs, L. (1957) *Nature* **179**, 360.
2. Adams, D. O. and Yang, S. F. (1979) *Proc. Natl Acad. Sci., U.S.A.* **76**, 170.
3. Ichihara, A., Shiraishi, K., Sato, H., Sakamura, S., Nishiyama, K., Sakai, R., Furusaki, A. and Matsumoto, T. (1977) *J. Am. Chem. Soc.* **99**, 636.
4. Ichihara, A., Shiraishi, K., Sakamura, S., Nishiyama, K. and Sakai, R. (1977) *Tetrahedron Letters* 269.

5. Ichihara, S., Shiraishi, K., Sakamura, S., Furusaki, A., Hashiba, N. and Matsumoto, T. (1979) *Tetrahedron Letters* 365.
6. Mitchell, R. E. (1985) *Phytochemistry* **24**, 1485.
7. Baldwin, J. E., Adlington, R. M. and Rawlings, B. J. (1985) *Tetrahedron Letters* **26**, 481.
8. Nakaparksin, S., Gil-Av, E. and Oro, J. (1970) *Analyt. Biochem.* **33**, 374.
9. Mitchell, R. E. and Young, H. (1985) *Phytochemistry* **24**, 2716.
10. Wakamiya, T., Wakamoto, H. and Shiba, T. (1985) *Tetrahedron Letters* 4411.
11. Pirrung, M. C. and McGeehan, G. M. (1986) *J. Org. Chem.* **51**, 2103.
12. Liu, H.-W., Achus, R. and Walsh, C. T. (1984) *J. Am. Chem. Soc.* **106**, 5335.