Phytochemistry, 1978, Vol 17, p 802 Pergamon Press. Printed in England

THE ABSOLUTE CONFIGURATION OF THE NEW AMINO ACID 2-AMINO-4-METHYL-HEX-5-ENOIC ACID FROM A NEW GUINEA *BOLETUS**

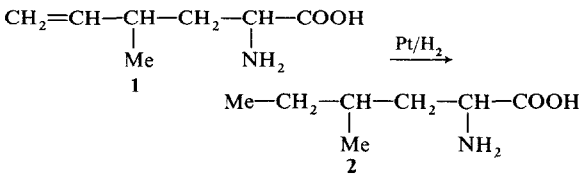
EMERY GELLERT, BERTHOLD HALPERN and RICHARD RUDZATS
Department of Chemistry, University of Wollongong, Wollongong, N.S.W. 2500, Australia

(*Received* 30 *November* 1977)

Key Word Index—*Boletus*; Boletaceae; amino acids; absolute configuration.

Abstract—The absolute configuration of the 2-amino-4-methyl-hex-5-enoic acid isolated from *Boletus* was shown to be 2*S*, 4*S*, by an unambiguous synthesis of its dihydro derivative from 2*S*-(−)-2-methylbutan-1-ol.

We have previously reported the isolation of 2-amino-4-methyl-hex-5-enoic acid (**1**) [1, 2] and shown that the asymmetric center at carbon-2 was *S* but could not establish the configuration at carbon-4 by physicochemical methods. We have now synthesized (2*S*,4*S*)-2-amino-4-methyl hexanoic acid (**2**) unambiguously from 2(*S*)-methylbutan-1-ol and acetylamino malonate [3, 4], followed by resolution of the carbon-2 center of the acetylaminomethylhexanoic acid by hog kidney acylase I [5, 6]. The synthetic product was identical in all respects with the dihydro compound (**2**) obtained from the natural product (**1**) by catalytic hydrogenation. As catalytic hydrogenation of the Δ5 double bond at room temperature will not change the chirality at C-4, the absolute configuratio of **1** is established as (2*S*,4*S*)-2-amino-4-methyl-hex-5-enoic acid.



The unambiguous synthesis of sterically pure **2** also establishes the stereochemistry of the naturally occurring amino acid, homoisoleucine, which had been isolated by Fowden and Smith [7] from *Aesculus californica*. Fowden *et al.* [8] had already proposed the 2*S*,4*S* configuration for homoisoleucine on the basis of a comparison of its solubility, ORD and CD with those of isoleucine.

EXPERIMENTAL

Mps are uncorr. Optical rotations were determined on an ETL-NPL automatic polarimeter.

Hydrogenation of 2-amino-4-methyl-hex-5-enoic acid (**1**). 0.1 g **1** over Pt required one mole of hydrogen (15.4 ml) and gave (2*S*, 4*S*) 2-amino-4-methylhexanoic acid (**2**) (0.1 g) mp 240–246°; *m/e* 146, MH^+ from CI; $[\alpha]_D^{22}$ +34.3° (*c* 0.385 in HOAc).

(2S,4S)-*2-Amino-4-methylhexanoic acid.* Refluxing 2*S*-(+)-1-bromo-2-methylbutane (4.5 g, prepared from 2*S*-(−)-2-methylbutan-1-ol) with ethyl acetamidomalonate (5.4 g) and NaOEt (0.6 g) in EtOH [3, 4] gave a mixture of (2*S*,4*S*) and (2*R*,4*S*) ethyl 2-acetamido-2-carbethoxy-4-methylhexanoate as a light yellow oil (5.6 g). Alkaline hydrolysis (10% NaOH, 2 hr), followed by refluxing (2 hr) of the acidified soln yielded a mixture of (2*S*,4*S*) and (2*R*,4*S*)-2-acetamido-4-methylhexanoic acid (2.3 g), mp 190–191° from H_2O, $[\alpha]_D^{27}$ +5.1° (*c*, 2.86 in MeOH). The CI (isobutane) MS showed peaks at *m/e* 188, MH^+, 142 (M^+-COOH), 130 (M^+-C_4H_9), 99 (M^+-COOH—MeCO), 57 $C_4H_9^+$. An aq. soln of the above acid mixture was adjusted to pH 7 with ammonia and hydrolysed at 38° with powdered hog kidney acylase I (12 mg) [5, 6] overnight. The soln was acidified to pH 5, filtered, passed through a Zeocarb 225 cation exchange resin column in the H^+ form, and the column washed with H_2O. The aq. eluates from the column were combined and evapd to dryness. Recrystallization of the solid from H_2O gave (2*R*,4*S*) 2-acetamido-4-methylhexanoic acid (0.4 g), mp 192°. $[\alpha]_D^{30}$ +22.3° (*c*, 6.27 in MeOH). Found: C, 58.0; H, 9.0; N, 7.3%; $C_9H_{17}NO_3$ requires: C, 57.7; H, 9.1; N, 7.5%. The amino acid was eluted from the column with 2N ammonia, and the soln concd to *ca* 200 ml. Cooling of the soln gave the lustrous, colourless crystals of (2*S*,4*S*)-2-amino-4-methyl-hexanoic acid (0.5 g), mp 240–244°. Found: C, 57.9; H, 10.4; N, 9.6%; $C_7H_{15}NO_2$ requires C, 57.9; H, 10.4; N, 9.6%. $[\alpha]_D$ +35.4° (*c*, 0.41 in glacial AcOH) and $[\alpha]_D^{22}$ +25.7° (*c*, 1.0 in 5 N HCl). The CI (isobutane) MS showed peaks at *m/e* 146 MH^+, 100 (M^+-COOH), 74 (M^+-C_5H_{11}) and 57 $C_4H_9^+$, identical with the acid obtained on hydrogenation of **1** (MS, GLC, TLC, mp, mmp). This is in agreement with data recently obtained by Bernasconi *et al.* [9] who used α-chymotrypsin for the resolution of the C-2 centre. The 2*S* configuration of our amino acid was also confirmed by GLC of its *N*-TFA-*L*-prolyl methyl ester derivative [10].

Acknowledgements—The authors are indebted to Dr E Challen of the University of N.S.W., Kensington for microanalyses, and to Dr J. A. Lamberton of CSIRO, Melbourne for the measurements of the rotations.

REFERENCES

1. Rudzats, R., Gellert, E. and Halpern, B. (1972) *Biochem. Biophys. Res. Commun.* **47**, 290.
2. Gellert, E., Halpern, B. and Rudzats, R. (1973) *Phytochemistry* **12**, 689.
3. Albertson, N. F. and Tullar, B. F. (1945) *J. Am. Chem. Soc.* **67**, 502.
4. Snyder, H. R., Shekleton, J. F. and Lewis, C. D. (1945) *J. Am. Chem. Soc.* **67**, 310.
5. Fodor, P. J., Price, V. E. and Greenstein, J. P. (1949) *J. Biol. Chem.* **178**, 503.
6. Fodor, P. J., Price, V. E. and Greenstein, J. P. (1950) *J. Biol. Chem.* **182**, 467.
7. Fowden, L. and Smith, A. (1968) *Phytochemistry* **7**, 809.
8. Fowden, L., Scopes, P. M. and Thomas, R. N. (1971) *J. Chem. Soc. (C)*, 833.
9. Bernasconi, S., Corbella, A., Gariboldi, P. and Jommi, C. (1977) *Gazz. Chim. Ital.* **107**, 95.
10. Halpern, B. and Westley, J. W. (1965) *Biochem. Biophys. Res. Commun.* **19**, 361.

* Part 3 in the series 'Constituents of a New Guinea *Boletus*'. For Part 2 see ref. [2].