Phytochemistry, 1975, Vol. 14, p. 1434. Pergamon Press. Printed in England.

CEREVISTEROL AND ERGOSTEROL PEROXIDE FROM *ACREMONIUM LUZULAE*

PAOLO CECCHERELLI, RENATA FRINGUELLI and GIAN FEDERICO MADRUZZA

Istituto di Chimica Organica, Facoltà Farmacia dell'Università Perugia, Italia

and

MARIO RIBALDI

Istituto di Micologia, Facoltà di Agraria dell'Università Perugia, Italia

(*Received* 30 *July* 1974)

Key Word Index—*Acremonium luzulae*; Moniliaceae fungi; cerevisterol; ergosterol peroxide.

Plant. Acremonium Luzulae (Fuckel) Gams.* A voucher specimen is deposited in the Istituto di Micologia, Facoltà di Agraria dell'Università, Perugia. *Previous work.* Characterization of several glycosidic diterpenes [1–3].

Present work. Cultures were extracted with BuOH and the residue was purified by SiO_2 column chromatography with C_6H_6–EtOAc. *First fraction*: ergosterol peroxide $C_{28}H_{44}O_3$, m.p. 182–184°, $[\alpha]_D$ −23·5° (EtOH). Ergosterol peroxide acetate, m.p. 199–201° [4]. M.p., TLC, IR, PMR and MS are identical with those of a pure sample prepared by synthesis [5]. *Second fraction*: cerevisterol (ergosta-7:22-diene-3β:5α:6β-triol) $C_{28}H_{46}O_3$ [6], m.p. 252–255°, $[\alpha]_D$ −80·5° (pyridine), MS *m/e* 412 (M^+ −18), 394, 376, 361, 269, 251, 69. It gives a diacetate $C_{32}H_{50}O_5$, m.p. 167–171°, $[\alpha]_D$ −147° (EtOH). All the analytical and spectroscopical data were identical with those of a pure sample prepared by synthesis [7].

* This fungus previously was classified as Oospora Virescens (Link) Wallr.

Acknowledgement—This investigation was supported by C.N.R., Italy.

REFERENCES

1. Cagnoli-Bellavita, N., Ceccherelli, P., Ribaldi, M., Polonsky, J. and Baskevitch, Z. (1969) *Gazz. Chim. Ital.* **99**, 1354.
2. Cagnoli-Bellavita, N., Ceccherelli, P., Mariani, R., Polonsky, J. and Baskevitch, Z. (1970) *European J. Biochem.* **15**, 356.
3. Ceccherelli, P., Cagnoli-Bellavita, N., Polonsky, J. and Baskevitch, Z. (1973) *Tetrahedron* **29**, 449.
4. Fattorusso, E., Magno, S., Santacroce, C. and Sica, D. (1974) *Gazz. Chim. Ital.* **104**, 409.
5. Windaus, A. and Brunken, J. (1928) *Ann. Chem.* **460**, 225.
6. Kocor, M. and Schmidt-Szalowska, A. (1972) *Bull. Acad. Pol. Sci. Ser. Sci. Chim.* **20** (6), 515.
7. Alt, G. H. and Barton, D. H. R. (1954) *J. Chem. Soc.* 1356.

Phytochemistry, 1975, Vol. 14, pp. 1434–1436. Pergamon Press. Printed in England.

L-γ-PROPYLIDENEGLUTAMIC ACID AND RELATED COMPOUNDS FROM *MYCENA PURA**

SHIN-ICHI HATANAKA and HIDEKI KATAYAMA

Department of Biology, College of General Education, The University of Tokyo, Komaba, Meguro-ku, Tokyo, Japan

(*Received* 5 *November* 1974)

Key Word Index—*Mycena pura*; Tricholomataceae; Basidiomycetes; L-γ-methyleneglutamic acid; L-γ-ethylideneglutamic acid; L-γ-Propylideneglutamic acid.

Mycena pura (Fr.) Kummer is one of the most common and widely distributed fungi. A PC survey revealed that the fruit bodies of this fungus contained several unusual amino acids. We have

now identified three which give brown colorations with ninhydrin. From their positions on 2D-chromatograms, one is γ-methyleneglutamic acid and the other two are the ethylidene and propylidene homologues. They were isolated by fractionation first on a column of Dowex 1 in acetate form, and subsequently with a cellulose column. L-γ-Methyleneglutamic acid was first isolated from young plants of *Arachis hypogaea* [1] and subsequently from some other legumes such as *Amorpha fruticosa* [2] and *Tetrapleura tetraptera* [3] from the Liliaceae, *Tulipa gesneriana* [4,5], *Lilium maximowiczii* [6], *Lilium candidum* [7] and from a fern *Phyllitis scolopendrium* [8]. The next higher homologue, L-γ-ethylideneglutamic acid was discovered from fruit capsules of *Tulipa gesneriana* [9]. This amino acid was reported to occur also in two legumes, *Tetrapleura tetraptera* [3] and *Guilandina crista* [10]. The identifications of these two amino acids from *Mycena pura* were based on elementary analysis, optical rotation and comparison of their IR spectra with those of the authentic specimens isolated from tulip. The evidence for the occurrence in nature of L-γ-propylideneglutamic acid, however, has not yet been reported.

The elementary analysis of the third amino acid isolated from this fungus was in good agreement with the formula $C_8H_{13}NO_4$, and on hydrogenation in the presence of Adams platinum catalyst it absorbed one mole hydrogen and changed to the substance, which gave a normal violet ninhydrin coloration. The R_f of the hydrogenation product was much higher than that of the original amino acid on cellulose-TLC with the solvent (a) as in the case of L-γ-methyleneglutamic acid and its saturated form. Additionally, the hydrogenation product was separated into two components of approximately equal amount on PC with the solvent (c) [11]. They correspond presumably to *threo*- and *erythro*-forms of γ-propylglutamic acid. The oxidation in diluted H_2SO_4 gave aspartic acid. These results showed that the third amino acid is γ-propylideneglutamic acid. The shift of $[\alpha]_D$ by the addition of HCl suggests that this amino acid belongs to the L-series. Finally the NMR spectrum was determined in D_2O and the results were consistent with the proposed structure.

EXPERIMENTAL

General. Mps are uncorrected. NMR spectra were recorded at 50° in D_2O with DSS as an internal standard. Evaporation of solvents was done with a rotary evaporator *in vacuo* below 40°.

Solvents for paper chromatography. Solvents used were *n*-BuOH–HOAc–H_2O (63:10:27) (a), PhOH–H_2O (in presence of NH_3 vapour) (25:9) (b) and upper layer of *t*-AmOH–HOAc–H_2O (20:1:20) (c) [11].

Isolation of amino acids. Fruit bodies of *Mycena pura* (Fr.) Kummer† (8·2 kg) were collected in late September 1973 in Nagano Prefecture and blended in 80% EtOH (×4). The combined, filtered extract (70 l.) was passed through a column of Amberlite 1R-120 (H^+) (1·5 l.). After the column was washed thoroughly with 80% EtOH and H_2O, successively, the absorbed amino acids were eluted with 2 N NH_4OH (20 l.). NH_3 was evaporated to give 60 g thick syrup, which was applied to a column Dowex 1 × 4 (40 × 1100 mm, $MeCOO^-$, equilibrated with 0·2 N HOAc) and eluted with 0·2 N HOAc. Though γ-ethylidene- and γ-propylideneglutamic acid appeared in the same fractions as glutamic acid, they could be separated from each other using a cellulose column (36 × 810 mm) and solvent (a). The fractions of each amino acid were extracted 4× with H_2O, the water layers combined and concentrated. γ-Methyleneglutamic acid was displaced from the Dowex 1-column between its higher homologues and aspartic acid. After concentration of relevant fractions, crude crystals separated. The yield of crude γ-methylene-, γ-ethylidene- and γ-propylideneglutamic acids were 4·48 g, 70 mg and 740 mg, respectively. Each sample was recrystallized 3× from H_2O.

L-γ-Methyleneglutamic acid. Pure crystals decomposed gradually above 180°. [Lit. 196° [1]] (Found: C, 45·30; H, 5·39; N, 8·60. Calc for $C_6H_9NO_4$: C, 45·28; H, 5·70; N 8·80%) $[\alpha]_D^{23}$ +17° (*c* 1, 3 N HCl), [Lit. $[\alpha]_D^{23}$ +14·0° (11% HCl) [12], $[\alpha]_D^{18}$ +12·8° (11% w/v HCl) [5]].

L-γ-Ethylideneglutamic acid. mp 171–2° (decomp.) [Lit. 198–201° [10]] (Found: C, 44·21; H, 6·81; N, 7·24. Calc for $C_7H_{11}NO_4 \cdot H_2O$: C, 43·98; H, 6·85; N, 7·33%). $[\alpha]_D^{23}$ +40° (*c* 1, 6 N HCl) [Lit. $[\alpha]_D^{20}$ +38·3° (*c* 1·4, 6 N HCl) [9], $[\alpha]_D^{25}$ +41·2° (*c* 1·5, 6 N HCl) [10]].

L-γ-Propylideneglutamic acid. mp 162°–3° (decomp.) (Found: C, 51·57; H, 6·88; N, 7·31. $C_8H_{13}NO_4$ requires: C, 51·33; H, 7·00; N, 7·48%). $[\alpha]_D^{23}$ +35° (*c* 1, H_2O); +60° (*c* 0·5, 3 N HCl). NMR: δ(ppm) 7·2 (*t*, γC=CH), 4·0 (*t*, αCH), 3·0 (*quar.*, βCH_2) 2·4 (*quin.*, γC=CH–CH_2) and 1·0 (*t*, Me). Degradation: 3·15 mg were dissolved in 1 ml 10% H_2SO_4, and to this soln 1·5 ml of 1% $KMnO_4$ were added dropwise. The reaction mixture was immediately centrifuged, the supernatant and washing were combined. After treatment with 1 ml Amberlite IR-120(H^+), the product was analysed with TLC and the solvents (a) and (b).

Acknowledgements—The authors wish to thank Dr. T. Hongo, Shiga University, for the identification of fungi, Dr. L. Fowden, Director of Rothamsted Experimental Station, for his kind supply of L-γ-methyleneglutamic acid and L-γ-ethylideneglutamic acid, each isolated from tulip and Dr. K.

* Part 11 in the series "Biochemical Studies on Nitrogen Compounds of Fungi". For Part 10 see Hatanaka, S.-I. and Niimura, Y., *Sci. Pap. Coll. Gen. Educat. Univ. Tokyo* (in press).

† The fungus was identified by Dr. T. Hongo, Shiga University. Voucher Specimens are deposited in the Department of Biology, College of General Education, The University of Tokyo.

Koyano, Department of Chemistry of our campus, for the determination of NMR. The kind help by Mmes. S. Takizawa, K. Moriguchi, Misses Y. Nagasaka, H. Taru and Mr. Soma in the collection of fungi is also greatly acknowledged.

REFERENCES

1. Done, J. and Fowden, L. (1952) *Biochem. J.* **51,** 451.
2. Tschiersch, B. (1962) *Phytochemistry* **1,** 103.
3. Gmelin, R. and Larsen, P. O. (1967) *Biochim. Biophys. Acta* **136,** 572.
4. Zacharius, R. M., Pollard, J. K. and Steward, F. C. (1954) *J. Am. Chem. Soc.* **76,** 1961.
5. Blake, J. and Fowden, L. (1964) *Biochem. J.* **92,** 136.
6. Ozawa, M., Inatomi, H., Suyama, Y. and Inukai, F. (1967) *Meiji Daigaku Nogakubu Kenkyu Hokoku* **22,** 7.
7. Rossetti, V. and Suria, M. (1972) *Phytochemistry* **11,** 859.
8. Virtanen, A. I. and Berg, A. (1955) *Acta Chem. Scand.* **9,** 553.
9. Fowden, L. (1966) *Biochem. J.* **98,** 57.
10. Nulu, J. R. and Bell, E. A. (1972) *Phytochemistry* **11,** 2573.
11. Gray, D. O., Blake, J., Brown, D. H. and Fowden, L. (1964) *J. Chromatog.* **13,** 276.
12. Nakayama, Y. and Kaneko, T. (1957) *J. Chem. Soc. Japan* **78,** 1216.

Phytochemistry, 1975, Vol. 14, p. 1436. Pergamon Press. Printed in England.

L-3-(3-CARBOXY-4-FURYL)ALANINE FROM *TRICHOLOMOPSIS RUTILANS*

SHIN-ICHI HATANAKA and YUKIO NIIMURA
Department of Biology, College of General Education, The University of Tokyo, Komaba, Meguro-Ku, Tokyo, Japan

(*Revised received* 4 *December* 1974)

Key Word Index—*Tricholomopsis rutilans*; Tricholomataceae; Basidiomycetes; L-3-(3-carboxy-4-furyl)alanine.

Doyle and Levenberg [1] reported recently a new amino acid L-3-(3-carboxy-4-furyl)alanine from *Phyllotopsis nidulans* (Pers. ex Fr.) Sing. Independently we also isolated the same amino acid from another fungus, *Tricholomopsis rutilans* (Fr.) Sing.* Identification was based on IR and TLC comparison with an authentic sample from *Phyllotopsis*.

EXPERIMENTAL

The amino acid fraction obtained from the fruit bodies (3 kg) was fractionated with a column of Dowex 1 (AcO^-) and 0·5 N HOAc as an eluting agent, giving pure fractions. Yield: 695 mg. Mp 215–6° (decomp.) $[\alpha]_D^{30}$ −48° (*c* 1, H_2O), −28° (*c* 0·5, 3 N HCl). UV: $\lambda_{max}^{H_2O}$ 239 nm (ε 2100), pH 4·3. IR (furan): 3135, 875, 803 and 772 cm^{-1}. NMR (in 5% DCl, DSS): δ 3·3 (*m*), 4·36 (*q*), 7·45 (*s*) and 8·05 (*d*, *J* 1·2 Hz).

Acknowledgement—We wish to thank Dr. R. R. Doyle, Denison University, for his generous supply of the amino acid of *Phyllotopsis nidulans*.

REFERENCES

1. Doyle, R. R. and Levenberg, B. (1974) *Phytochemistry* **13,** 2813.

* Vouchers are deposited in the Department of Biology, College of General Education, The University of Tokyo.

Phytochemistry, 1975, Vol. 14, pp. 1436–1437. Pergamon Press. Printed in England.

MAIN FLAVONOIDS IN NEEDLES OF *LARIX DECIDUA**

GERARD J. NIEMANN
Botanical Laboratory, University Utrecht, The Netherlands

(*Received* 10 *September* 1974)

Key Word Index—*Larix decidua*; Pinaceae; Gymnospermae; flavonoids.

Plant. Larix decidua Mill. Voucher specimen No. GN3, Institute for Systematic Botany, University Utrecht. *Source.* Arboretum Schovenhorst, Putten, The Netherlands, Aug. 1973. *Previous work on leaves.* Lipids [1], sterols [2], *O*-methylinositols [3], and organic acids [4].