Phytochemistry, 1973, Vol. 12, pp. 689 to 692. Pergamon Press. Printed in England.

AMINO ACIDS AND STEROIDS OF A NEW GUINEA *BOLETUS**

E. GELLERT, B. HALPERN and R. RUDZATS

Chemistry Department, Wollongong University College, Wollongong, N.S.W. 2500, Australia

(*Received* 5 *September* 1972. *Accepted* 5 *October* 1972)

Key Word Index—*Boletus*; Boletaceae; amino acids; steroids; ergosterol; mannitol; GC–MS.

Abstract—In addition to the previously reported isolation of L-2-amino-4-methylhex-5-enoic acid, eleven known amino acids, mannitol, ergosterol, and two new steroids were isolated from an allegedly hallucinogenic *Boletus*. A modification of the GC–MS method used for the identification of neutral α-amino acids is also reported.

INTRODUCTION

COMPOUNDS of fungal origin, e.g. psilocybine and psilocine isolated from 'teonanacatl' the sacred mushrooms of Mexico,[1,2] gained importance several years ago because of their hallucinogenic properties[3,4] are similar to those of mescaline and lysergic acid derivatives. As it was claimed that some Australian[5,6] and New Guinean[7–9] mushrooms also cause hallucinations, we investigated these species. But the activity of *Panaeolus ovatus*, the suspected Australian species, was, in fact, found to be due to the presence of *Psylocybe cubensis*[10,11] in the field collections. Hallucinatory activity of a different type[12] was, however, ascribed to the New Guinean mushrooms as they cause multiple or inverted vision. This activity commences about 1 hr after ingestion (raw or cooked) and lasts for several hours, depending on the amount of mushrooms consumed. Full mental control is however not regained for several days. 'Namanama', the mushroom responsible, was tentatively identified[13] (Botany Department, University of Queensland, Brisbane) as *Boletus*, section

* Part II in the series "Constituents of a New Guinea *Boletus*". For Part I see *Biochem. Biophys. Res. Commun.* **47**, 290 (1972).

[1] R. SINGER, *Mycologia* **50**, 239 (1958).
[2] R. HEIM and R. G. WASSON, *Les Champignons Hallucinogénes Du Mexique*. Editions Du Muséum National d'Histoire Naturelle, Paris (1958).
[3] A. HOFMANN, R. HEIM, A. BRACK and H. KOBEL, *Experientia* **14**, 107 (1958).
[4] V. LOPPINET, *Bull. Soc. Pharm. Nancy* **81**, 18 (1969).
[5] J. B. CLELAND, *Toadstools and Mushrooms and other Larger Fungi of South Australia*, Government printer, Adelaide (1934).
[6] J. H. WILLIS, *Victorian Toadstools and Mushrooms*, 2nd Edn, Field Naturalists Club of Victoria, Melbourne (1957).
[7] W. A. ROSS, *Anthropos* **31**, 351 (1936).
[8] G. F. VICEDOM and H. TISCHNER, *Die Mbowamb. Die Kultur der Hagenberg-Stämme im Östlichen Zentral Neuguinea*, Museum für Völkerkunde, Hamburg (1943–48).
[9] A. L. GITLOW, *Economics of the Mt. Hagen Tribes, New Guinea*. Monograph 12, American Ethnographical Society, New York (1947).
[10] J. E. C. ABERDEEN and W. JONES, *Austral. J. Sci.* **21**, 149 (1958).
[11] D. F. FRANCIS and R. V. SOUTHCOTT, *Plants Harmful to Man in Australia*. Misc. Bull. No. 1, Botanic Gardens, Adelaide (1967).
[12] J. S. THORPE, Mt. Hagen, New Guinea, personal communication.
[13] J. E. C. ABERDEEN, University of Queensland, Brisbane, unpublished data.

Ixocomus, group *Nudi* and was collected in the Mt. Hagen area during 1963. Pharmacological tests used for the detection of LSD25, mescaline, or psilocybine type activity were negative.[14] Other tests to establish the exact nature of the physiological activity are in progress.

RESULTS AND DISCUSSION

The creamy yellow mushroom with a smooth purple cap grows under acorn trees and has no ring or network on its stem. The pores bruise easily on handling when the cream colour turns to ink-blue for a short time. The methanolic extract—which deposited crystalline mannitol on concentration—was distributed between water and light petrol. After freeing the aqueous portion from carbohydrates, pigments and plant acids, the amino acids (2·5% dry wt) were identified by PC, by the use of an automatic amino acid analyser, and with the help of a new modification of the GLC and MS analysis procedure described by Jellum.[15] The employment of Brenner's thionyl chloride–ethanol reagent[16]—instead of the customary ethanol hydrochloric acid—avoided water formation during esterification while condensation of the amino group with pivaldehyde (2,2-dimethylpropanal) increased the thermal stability of the amino acid esters. (Threonine gives a cyclic derivative with pivaldehyde.[17])

TABLE 1. CHARACTERISTIC MS FRAGMENTS OF *N*-NEOPENTYLIDENE AMINO ACID ETHYL ESTERS

Amino acid	M^+	M^+-Me	M^+-CMe_3	M^+-COOEt
Alanine	185	170	128	112
Glycine	—	156	114	98
Valine	213	198	156	140
Leucine / Isoleucine	—	212	170	154
Threonine	—	—	—	142
L-2-Amino-4-methylhex-5-enoic acid	*	224	182	166
Methionine	245	—	188	172

* Gives an $M^+ + 1$ peak.

The fragmentation patterns in the mass spectrum of the open-chain neopentylidene amino acid esters show the presence of characteristic peaks at M^+-15, M^+-57, and M^+-73 m.u. (Table 1) which allows identification of the individual components in conjunction with their respective GLC retention times (Table 2). The absence of peptides and proteins in the extract was shown by the identity of the automatic amino acid analyser data before and after hydrolysis of the aqueous extract.

The light petrol. solution gave, after chromatography on silica, three steroidal components: Ergosterol and small quantities of two new steroids. *Steroid A*, m.p. 109–110°, has an *m/e* 380 molecular ion peak showing the presence of 27 carbon and 1 oxygen atoms in the molecule. Its UV spectrum is superimposable on that of ergosterol indicating the presence of the same conjugated double bond system in both molecules. The IR spectrum

[14] J. SCHMUTZ, Bern, personal communication.
[15] E. JELLUM, V. A. BACON, W. PATTON, W. PEREIRA, JR. and B. HALPERN, *Anal. Biochem.* **31**, 339 (1969).
[16] M. BRENNER and W. HUBER, *Helv. Chim. Acta* **36**, 1109 (1953).
[17] W. KLYNE, Z. BADR, R. BONNETT and T. R. EMERSON, *J. Chem. Soc.* 4503 (1965).

showed the absence of hydroxyl groups, the presence of a keto group (1740 cm^{-1}) and a Δ22 double bond (970 cm^{-1}) indicating a nor-ergosterone structure. *Steroid B*, m.p. 179–180°, had the molecular formula $C_{28}H_{44}O_3$, which formally represents the addition of two oxygen atoms to ergosterol. Its low R_f (0·12) and IR absorption around 3400 cm^{-1} shows the presence of hydroxyl groups in the molecule, one of which is in the 3β-position (1045 cm^{-1}). The absence of conjugated double bonds are shown by the lack of UV absorption above 220 nm, while IR absorption bands at 803, 838 cm^{-1} and 1660, 970 cm^{-1} indicate the presence of Δ5 and Δ22 double bonds. Elucidation of the above structures must, however, await further investigation and a new collection.

TABLE 2. GLC RETENTION TIMES OF *N*-NEOPENTYLIDENE AMINO ACID ETHYL ESTERS*

Amino acid	R_t (min)	Temp. (°)	Amino acid	R_t (min)	Temp. (°)
Alanine	11·0	160	Threonine	15·8	198
Glycine	11·6	165	L-2-Amino-4-methylhex-5-enoic acid	16·7	205
Valine	13·2	178	Methionine	20·8	238
Leucine / Isoleucine	14·9	191			

* Measured in a Packard–Becker 409 Gas Chromatograph on a 182 × 25 cm stainless steel column packed with 5% OV17 on Chromasorb W (silanized) programmed from 80° at 8°/min with a 20-ml/min flow-rate of nitrogen carrier gas.

EXPERIMENTAL

Extraction. Finely milled air-dried mushrooms (1·27 kg) were extracted by percolation with MeOH at room temp. On concentration crude crystals of mannitol (R_f, m.m.p., hexa-acetate) separated. After evaporation of the extract, the syrupy residue was taken into 1 l. H_2O and extracted with light petrol. (b.p. 40–60°). The aqueous layer yielded a viscous syrup (A), while the petroleum extract gave a dark yellow gum (B).

The amino acids. The hydrolysate obtained from *A* with H_2SO_4 gave, on an automatic amino acid analyser, the same qualitative result as untreated *A*. Further separation by PC gave twelve amino acids. L-2-Amino-4-methylhex-5-enoic acid (0·04%),[18] m.p. 240–242° (decomp.), from aq. EtOH, R_f 0·80 in BuOH–AcOH–H_2O (2:1:1), 0·87 in Ph–OH–H_2O; leucine and isoleucine (1·1%). Valine (0·3%); methionine (0·1%); tyrosine (0·05%); alanine (0·6%); threonine (0·15%); glycine (0·1%); arginine, lysine and histidine (0·1%). The above amino acids with the exception of tyrosine and the basic amino acids were identified by PC, and automatic amino acid analyser in addition to the R_ts in GLC and GC–MS of their *N*-neopentylidene derivatives.

Preparation of the N-*neopentylidene derivative.* The amino acid (10 mg) was esterified with EtOH–$SOCl_2$ (10:1, 0·1 ml). The residue, after removing excess reagent, was dissolved in 50 μl. EtOH and buffered to pH 8 with ion-exchange beads. Pivaldehyde (10 μl.) was added and the reaction mixture was allowed to stand, over molecular sieve, for 30 min. The final product does not require purification. Tyrosine and the basic amino acids (0·1%) were identified by 2-D PC and the automatic amino acid analyser.

The steroids. The light petrol. extract *B* (6·5 g) was chromatographed on 300 g Silica in benzene–acetone (19:1). *Steroid A*, R_f 0·96, m.p. 109–110° from C_6H_6–MeOH, gave positive Liebermann–Burchard, Salkowski and Rosenheim tests. The MS showed peaks at *m/e* 380 (molecular ion), 378, 363, 253, 237, 213, 199, 185, 171, 157, 143, 129, 98, 73, 69, 60, 57, 55, 44 and 43. γ_{max} (Nujol) 1740, 1220, 1175, 970 cm^{-1}.

Ergosterol[19] was isolated from the following fraction. Its identity was established by direct comparison with authentic material (IR, UV, MS, TLC and acetate). *Steroid B*, R_f 0·12, m.p. 179–180° from MeOH, was isolated from the last fractions. (Found: C, 78·4; H, 10·3. $C_{28}H_{44}O_3$ requires: C, 78·5; H, 10·4%.) It gave positive Liebermann–Burchard and negative Tortelli–Joffe tests. The MS showed peaks at *m/e* 428 (molecular

[18] R. RUDZATS, E. GELLERT and B. HALPERN, *Biochem. Biophys. Res. Commun.* 47, 290 (1972).
[19] C. W. SHOPPEE, *Chemistry of the Steroids*, 2nd Edn, Butterworth, London (1964).

ion) and 410, 392, 305, 304, 303, 251, 81, 69, 57, 55 and 43. γ_{max} 3540, 3410, 1660, 1155, 1080, 1045, 970, 940, 860, 838, 803 cm^{-1}.

Acknowledgements—The authors are indebted to Mr. J. S. Thorpe (Territories of Papua and New Guinea) for collection of the mushrooms, Messrs. P. Nichols and L. James (C.S.I.R.O., Melbourne) for the automatic amino acid analyser data and the Australian Microanalytical Service, C.S.I.R.O., Melbourne, for the microanalysis.