Notes

Subsidiary Constituents from *Amanita Muscaria*[1a,b]

A. V. Subbaratnam and William B. Cook

Department of Chemistry, Montana State College, Bozeman, Montana

Received August 25, 1962

Review articles[2-4] record chronological accounts of the chemistry of muscarine, whose structure finally has been elucidated. However, the chemical basis for the psychoactive principles of the mushroom, *Amanita muscaria*, continues to baffle investigators. It is generally agreed that intoxication by the mushroom is due primarily to excitation of the central nervous system and that muscarine cannot possibly be responsible for the observed activity.[5] Investigators have labeled the mushroom's elusive hallocinogen as "Pilzatropine" for ease of reference.[6]

Hyoscyamine[7] and bufotenine[8] have been reported to be found in *Amanita muscaria*. These could, if present, account for the activity of the drug. However, such claims have not been substantiated by other investigators in the field.[6,9,10] Eugster[3] reported the absence of hyoscyamine and bufotenine in *A. muscaria* in the course of intensive investigations. He detected a labile substance which had a spectrum similar to that of the 4-hydroxyindole derivative, psilocybin, the psychoactive principle of the Mexican fungus, *Psilocybe mexicana*.[11]

A systematic chemical investigation of *A. muscaria* was initiated in this laboratory in an attempt to identify the psychoactive principles. Fractionation was carried out with four successive batches of mushroom,[12] taking precautions to prevent the formation of artifacts and progressively making changes in procedure based on data from biological tests. Details of this work will be reported later. A summary of the extraction procedure is given here.

Fresh, whole mushrooms, preserved in methanol, were filtered by draining through muslin cloth. The swollen mushrooms (40.5 kg.) were pulverized in a meat grinder and the pulp was spun as dry as possible in a basket centrifuge. The combined filtrates from draining and centrifuging were concentrated in a low temperature vacuum still. The concentrate, fraction I, was a thick, dark brown sirup (3.6 kg., pH 5.1).

The residual mushroom pulp (11.4 kg.) was dried in a current of air at 35°. The dried material was thoroughly extracted at room temperature with absolute methanol. The combined extracts were concentrated in a low temperature vacuum still. Fraction II was a light brown sirup (2.9 kg., pH 7.5).

The "marc" from the two previous extractions was further extracted at room temperature 3 times with pyrogen-free distilled water. The aqueous filtrates were concentrated in a low temperature vacuum still. Fraction III was a dark brown viscous solution (3.3 kg., 1% solids).

Biological testing (Table I) indicated that aqueous extract III was relatively free from muscarinic effects characteristic of fractions I and II. Methanolic extracts I and II were subjected to extensive solvent fractionation and chromatographic separation. The several resulting fractions which included minor crystalline products and a rather large essential oil fraction were subjected to biological screening. None exhibited significant "sympathotonia," the hyperpyrexia-mydriasis drug syndrome characteristic of LSD-25.

Table I

Biological Activities of Fractions[a]

	ED-50 mg./kg.[b]		
Symptoms	Fraction I ± SE	Fraction II ± SE	Fraction III ± SE
Ataxia of gait	2.15	1.65	..
Hypomatility	1.48	1.50	0.29 0.39
Loss of righting reflex	2.32 0.07	2.19 0.09	..
Prone position	2.15	..	..
Miosis	1.03	1.38 0.18	..
Salivation	1.04 0.56	1.02 0.18	..
Lacrimation	1.04 0.56	2.40	..
Hyperpyrexia	1.01	2.40 0.13	1.53
Slowing of respiration	1.01	1.63	0.40 0.24
Convulsions	2.39 0.10	2.40 0.13	..
Death	2.23 0.07	2.25 0.14	>0.65<0.90
Opisthotonos	2.32 0.07	2.37	..
Proptosis	2.15	..	..
Hypotonia	..	1.71	..
Mydriasis	..	..	1.5 0.15

[a] The values listed are the log ED_{50}. The extracts were administered intravenously to rabbits. [b] The ED_{50}'s were calculated using the method of Litchfield and Fertig. Insufficient data were available to determine the SE where this value is missing, the dose −50 and slope were estimated using probits of 0.1 and 99.99%, respectively, at the maximum 0% level and the minimum 100% level.

As a result of these findings the ultimate water soluble portion of the mushroom, freed from alkaloids, proteins, lipids and inorganic constituents, such as KCl, was considered to be the "active" fraction. Small doses of this fraction, comparable to those employed for LSD-25, produced in test animals mydriasis accompanied by hyperpyrexia. Whether the hypothetical "pilzatropine" or some such single constituent is responsible for the activity of the water concentrate or whether the hallucinogenic activity of the mushroom is due to a combined drug syndrome will have to be proved by human experiments in view of the manifestation of various pharmacological effects by the mushroom which are not always easily distinguishable and which require extreme caution in interpretation. It should be mentioned that hyperpyrexia *per se* need not necessarily produce nor be accompanied by mydriasis or

(1) (a) A preliminary report of this work was presented before the IUPAC Symposium on the Chemistry of Natural Products, Melbourne, Australia, August, 1960. (b) This investigation was supported in part by the Medical Sciences Research Foundation, Stanford University.

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(5) V. P. Wasson and R. G. Wasson, "Mushrooms, Russia and History", Vol. 1, Pantheon Books, New York, N. Y. 1957, pp. 190–214.

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(8) T. Wieland and W. Motzel, *Ann.*, **581**, 10 (1953).

(9) V. Kwasniewski, *Deut. Apoth-Ztg.*, **94**, 1177 (1954).

(10) L. R. Brady and V. E. Tyler, Jr., *J. Am. Pharm. Assn.* (Sci. Ed), **48**, 417 (1959).

(11) A. Hofmann, A. Frey, H. Ott, Th. Petrizilka, and F. Troxler, *Experientia*, **14**, 397 (1958).

(12) Authentic samples of *Amanita muscaria* were supplied by the Mycological Society of San Francisco.

miosis. For example, fumaric acid isolated from *A. muscaria* produced hyperpyrexia but neither mydriasis nor miosis in experimental animals. Therefore, in the biological screening of various fractions from *A. muscaria*, a material which produced mydriasis accompanied by hyperpyrexia was considered tentatively to possess LSD-like behavior.

Through fractionation of the aqueous concentrate (III) with judicious mixtures of methanol, ethanol, ethyl acetate, and acetone an hygroscopic crystalline material, provisionally designated "z" was isolated (307 mg., m.p. 135–138° dec).

Anal. Calcd. for $C_{15}H_{30}K_4O_{15}$: C, 29.7; H, 4.98; K, 25.77. Found: C, 30.17, 30.30; H, 4.83, 4.60; K, 25.23; mol. wt., 615; equiv. wt., 218 (potentiometric titration).

The analytical data are consistent with a compound having four COOK and three COOH groups. The n.m.r. spectrum showed only two peaks; one characteristic of methyl-keto functions, the other of labile hydrogens. Integration of these peaks suggests a ratio of three methyl groups to two labile hydrogens.

The very small amount of "z" left after pharmacological and analytical experiments was methylated with diazomethane. Its n.m.r. spectrum was complicated and showed no expected relationship to that of the original compound other than to confirm the methylation.

"z" was tested intravenously in rabbits at two-dose levels. A low level dose of "z," 1.0 mg./kg., resulted in mydriasis, 30 min. after administration, which persisted for 30 min. Pupil dilation was 2 mm. above normal. Three hr. after administration, there was a temperature rise of 0.9° which lasted less than 1 hr. At the high level dose of 6.25 mg./kg., mydriasis was immediate, persisting for 30 min. Pupil dilation was initially 1.5 mm. above normal, increasing to 2 mm. later. Thirty min. after administration, temperature rose 0.7° and persisted for 30 min. Fifteen min. after administration, there was rapid respiration persisting for 2 hr. There were no other symptoms.

By means of thin-layer chromatography minute quantities of indolic compounds have been isolated from the aqueous concentrate. From fractions I and II potassium gluconate, a glucoside m.p. 106° dec and two minor crystalline substances, m.p. 172–174° and 160–165°, respectively, were isolated as hitherto unreported constituents. Mannitol, fumaric acid, a fixed oil fraction, and a considerable amount of KCl also were isolated.

The fixed oil was fractionated on alumina columns and subjected to chemical and biological testing. All fractions were found to be mainly esters of oleic acid and devoid of insecticidal properties.[13]

Acknowledgment.—We wish to thank Dr. Edward Pelikan and his colleagues for carrying out the pharmacological tests, Varian Associates for n.m.r. data and Abbott Laboratories for large scale extractions.

(13) *A. muscaria* is commonly known as fly-agaric due to its reputed property as an insecticide.

Potential Carcinolytic Agents. I. Derivatives of P,P-Bis(1-aziridinyl)phosphinic Amide[1]

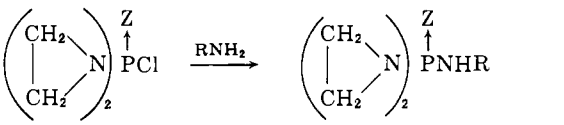
ORRIE M. FRIEDMAN, ROBERT S. LEVI, ZINON B. PAPANASTASSIOU,[2] AND WILSON M. WHALEY

Arthur D. Little, Inc., Acorn Park, Cambridge 40, Massachusetts

Received July 11, 1962; revised manuscript received February 2, 1963

The alkylating ability of aziridine is known to be enhanced by an electron-withdrawing N-substituent, and there are indications that molecules with several activated aziridine groups are likely to display antitumor activity.[3] However, a favorable therapeutic index depends on the entire molecular structure rather than simply on the cytotoxic moieties. A degree of selectivity of action has been achieved with certain P,P-bis(1-aziridinyl)phosphinic derivatives, notably the "dual antagonists."[4]

Further work along these lines has led to the preparation of the derivatives (II) reported in the present paper.



I

Ia, Z = O
Ib, Z = S

II

IIa, R = C_6H_{11}; Z = O
IIb, R = C_6H_{11}; Z = S
IIc, R = $(CH_2)_3OH$; Z = O
IId, R = $(CH_2)_3OH$; Z = S
IIe, R = $(CH_2)_3OCH_3$; Z = S

The usual procedure (excepting one instance)[5] for preparing P,P-bis(1-aziridinyl)phosphinic amides has been to attach the sensitive aziridine groups in the last step.[6] This approach was not used in our work because a number of the intermediates would contain reactive functional groups so situated that unwanted cyclizations could occur prior to the final step. The desired compounds were prepared from the intermediate P,P-bis(1-aziridinyl)phosphinic chloride (Ia) and the thiono analog (Ib), both of which were used directly without isolation. The phosphinic chloride was so unstable that it was used immediately upon preparation, whereas the thiophosphinic chloride could be kept at least overnight in solution at low temperatures. All reactions were conducted in the cold, and the products isolated under the mildest possible conditions because of their inherent instability. Simple amines such as cyclohexylamine, used in excess to serve as condensing agents, gave fairly good yields of nearly pure products (generally the yields decreased rapidly with attempts at purification). With other amines where triethylamine was used as the condensing agent the products were less easily purified. The oily products in particular were difficult to purify because they decomposed when subjected to most standard purification techniques, including molecular distillation at 10^{-6} mm. pressure or chromatography.

Pure derivatives could not be obtained from 1,3-propanediamine, 3-mercapto-1-propylamine, 1,3-dimercaptopropane, 3-mercapto-1-propanol, 1,3-propanediol, and ethyl glycinate. The use of sodium or magnesium salts of the alcohols and mercaptans was unsuccessful as was the triethylamine technique. In addition to triethylamine, various other hindered and unhindered amines (N-methylpiperidine, N-methylmorpholine, tri-

(1) This work was sponsored by the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Contract No. SA-43-ph-4360.

(2) To whom inquiries should be addressed.

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