Muscarine in *Clitocybe* Species

By K. GENEST, D. W. HUGHES, and W. B. RICE

Results of chemical and bioassays for muscarine in a sample of *Clitocybe dealbata* indicate the absence of other muscarine-like substances or muscarine potentiators. Evidence was found for the presence of muscarine in herbarium samples of *C. illudens*, *C. cerussata*, *C. dealbata*, and *C. rivulosa*. The age of samples ranged from 12 to 26 years.

A PREVIOUS COMMUNICATION (1) reported the isolation and determination of muscarine in *Clitocybe dealbata* and qualitative results on the physiological action of mushroom preparations injected into rats. It was of interest to know whether comparable results for muscarine content could be determined by chemical and biological assay, since muscarine-like potentiating factors have been reported in some species of *Inocybe* (2, 3). Other *Clitocybe* species have frequently been described as being toxic or of doubtful edibility (4–8). A collection of herbarium samples of such species was also examined for muscarine content.

EXPERIMENTAL

Material and Methods

Clitocybe dealbata—1965 crop: 57 Gm.; 1966 crop: 1.5 Gm.[1] of dry powder, passing 100-mesh sieve. Both samples from same location in Ottawa, Ontario.

Herbarium Samples—Samples were obtained from the Herbarium, Taxonomy and Mycology Section, Plant Research Institute, Department of Agriculture, Ottawa, Ontario. Relevant authenticative data are listed in Table I.

Chemical Determination—Muscarine was determined in accordance with the method described previously (1). For herbarium samples 0.25–0.5 Gm. of dried mushroom powder was used.

Biological Examination—The bioassay method of Malone *et al.* (9) was applied to the 1965 sample of *C. dealbata*. The standard for the bioassay was *dl*-muscarine iodide (Geigy). Healthy, nonfasted, adult male albino rats of the Wistar strain were used.[2] Muscarine was injected intraperitoneally into three groups of 10 rats each at 0.0125, 0.025, and 0.05 mg./Kg. (calculated as *l*-muscarine, free base). Mushroom preparations containing 27.3, 54.7, and 109.4 mg. of mushroom powder (100 mesh) per 15 ml. were injected into three groups of 10 rats each (1 ml./200 Gm.). Agar (0.25%) in water was used as dosage vehicle. Scores of the salivation metameter, as defined by Malone *et al.* (9), were measured. Preparations from herbarium samples were injected in amounts shown in Table II.

TABLE I—HERBARIUM SAMPLES OF *Clitocybe* SPECIES

Herbarium No.	Botanical Name	Common Name	Place and Time of Collection
DAOM 11834	*Cantharellus aurantiacus* (Wulf.) Fr. = *Clitocybe aurantiaca* (Fr.) Studer	Orange-yellow Chanterelle	Sept. 24, 1944, Chalk River, Ontario
DAOM 55728	*Clitocybe cerussata* var. *difformis* (Fr.) sensu Bres.	...	July 24, 1949, Yukon Territory
DAOM 33896	*Clitocybe dealbata* (Sow. ex Fr.) Kummer	Ivory clitocybe	Oct. 18, 1951, Vancouver, British Columbia
DAOM 10018	*Clitocybe illudens* Schw.	Jack-o'-lantern	Sept. 2, 1940, Oakville, Ontario
DAOM 24168	*Clitocybe illudens* Schw.	Jack-o'-lantern	Aug. 17, 1950, Ottawa, Ontario
DAOM 113652	*Clitocybe illudens* Schw.	Jack-o'-lantern	Aug. 10, 1954, Kentville, Nova Scotia
DAOM 64791	*Clitocybe rivulosa* (Pers. ex Fr.) Kummer	...	Sept. 16, 1946, Uppsala, Sweden

RESULTS AND DISCUSSION

Comparison of Chemical and Bioassays for C. dealbata—The muscarine content of the 1965 sample of *C. dealbata* was found to be 0.18% (0.16–0.21)[3] ($N = 16$) as determined by the chemical method (1) and calculated as *l*-muscarine (free base). The statistical analysis of the bioassay results is given in Table III. By this method, the sample was found to contain 0.26% (0.23–0.30)[3] (30 rats) of muscarine, also calculated in terms of the naturally occurring isomer. The λ value for this assay was found to be 0.2308 which agrees well with that reported by Malone *et al.* (9). The chemical and bioassays are in general agreement and the difference between the two is probably not due to the presence of other muscarine-like agents and/or potentiators, reported to occur in *Inocybe* species (2, 3), for which values obtained by bioassays were 3 to 13 times higher than those obtained by chemical assays. This interpretation is strengthened by the fact that the authors used *dl*-muscarine as standard and

Received July 10, 1967, from Research Laboratories, Food and Drug Directorate, Ottawa, Ontario, Canada.
Accepted for publication September 19, 1967.
The authors thank Dr. D. B. O. Savile, Plant Research Institute, Department of Agriculture, Ottawa, for herbarium samples, Dr. Leo Levi for encouragement, and Messrs. G. Bélec and W. Skakum for technical assistance.

[1] 1966 was an extremely poor year for *C. dealbata* in our region.

[2] From the animal colony of the Food and Drug Directorate.

[3] 95% confidence limits for the percentage figure.

TABLE II—MUSCARINE ASSAY AND BIOLOGICAL TESTS OF *Clitocybe* SAMPLES

Sample	Age, Yr.	Chemical Assay, % of Dry Wt.	Biological Tests: Amt. of Mushroom Used, mg./ml.	Biological Tests: Reaction
C. aurantiaca	$22^1/_2$	0.00	200/3	No reaction
C. cerussata	$17^1/_2$	0.012	200/4	Slight to moderate salivation and lacrimation
C. dealbata	$15^1/_3$	0.01	100/3	Moderate to profuse salivation, slight to profuse lacrimation, chromodacryorrhea
C. dealbata	$1^1/_4$	0.18	[a]	
C. dealbata	$^1/_4$	0.083	...	
C. illudens	$26^1/_2$	0.0075	150/3	Slight salivation ...
			450/7.5[b]	moderate salivation, slight lacrimation
C. illudens	$16^1/_2$	0.005	200/3	No reaction
C. illudens	$12^1/_2$	0.00	200/3	No reaction[c]
C. rivulosa	$20^1/_2$	0.035	30/3	Moderate to profuse salivation, moderate to profuse lacrimation, chromodacryorrhea

[a] Sample was bioassayed, see text. [b] 1.5, 2.25, and 3.75 ml. injected per rat. [c] One rat showed slight salivation 30 min. after injection.

TABLE III—ANALYSIS OF VARIANCE AND FACTORIAL ANALYSIS OF BIOASSAY

Term	*df*	*SS*	*MS*	*F*	*P*
Between treatments	5	176.588	35.317	14.468	<0.001
S vs. U	1	0.104	0.104	0.042	>0.20
Slope	1	166.057	166.057	68.028	<0.001
Departure from parallel	1	3.906	3.906	1.600	>0.20
Curvature	1	1.519	1.519	0.622	>0.20
Opposite curvature	1	5.002	5.002	2.049	0.10–0.20
Residual error	54	131.825	$s^2 = 2.441$		
Total	59	308.413			

calculated results for the bioassay on the basis that the racemic mixture has half the activity of naturally occurring *l*-(+)-muscarine (10). Some pharmacological studies, however, indicate that this is only an approximation (11).

Herbarium Samples—Various *Clitocybe* species have been classified as poisonous or of doubtful edibility. The older mycological literature can be confusing, mainly due to nonuniformity of nomenclature (12). *C. dealbata*, for instance, was classified without qualification as edible (4). Recent sources, however, list *C. dealbata*, *C. illudens*, *C. rivulosa* (6, 7), and *C. cerussata* var. *difformis* (7) as poisonous, and *C. aurantiaca* (6) as a mushroom of possible toxicity. Herbarium samples of these species were investigated for the presence of muscarine by the chemical method. Results of these tests and of the investigation of two recently collected samples of *C. dealbata* are given in Table II. Muscarine could be detected in all species which are considered poisonous. Two samples of *C. illudens* were muscarine positive, but the alkaloid was not detected in a third specimen. *C. aurantiaca* was negative also. The classification of this mushroom appears to be controversial, and while it is considered poisonous by some mycologists, others consider its edibility established (6, 13). Farnsworth (14) pointed out that the age of herbarium samples limits the usefulness of chemical tests, but the same author cites cases where 1300-year-old plants were still found to give positive alkaloid tests. A decline of muscarine content with time in herbarium samples is suggested by the results on samples 3 months, 15 months, and 15 years old (Table II). Yet compositional differences have also been observed between crops of the same year. Among the herbarium samples investigated *C. rivulosa* is the *Clitocybe* species exhibiting the highest potency, a finding which confirms recent work on surface-grown cultures of that species (15, 16). Results of chemical tests carried out on the herbarium samples were confirmed by biological tests (Table II). Since very little herbarium material was available, bioassays could not be performed. Qualitative muscarine symptoms in rats were afforded by all samples containing 0.0075% or more of muscarine.

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Keyphrases

Alkaloids—*Clitocybe dealbata*
Muscarine—isolation
Biological analysis—muscarine
Chemical analysis—muscarine
Clitocybe species—muscarine, aging effect

Fluorometric Determinations Comparable to Eye Response

By JOSEPH E. SINSHEIMER, JAMES T. STEWART*, and JOSEPH H. BURCKHALTER

A filter-photomultiplier tube system approaching the spectral response of the human eye has been selected, its spectral response recorded, and the system evaluated with a series of model fluorescent compounds.

FLUORESCENT tagging agents are being synthesized in these laboratories for potential use in fluorescent antibody-antigen techniques (1). To determine more rationally structures to be synthesized, it was desirable to direct these studies by an objective and sensitive comparison of fluorescence. Cognizant that the antibody-antigen technique ultimately depended upon visual observation of fluorescence, a filter-photomultiplier tube detection system that approached the spectral response of the human eye (2, 3) was developed for use with commercially available fluorometers. As this system has general application for the comparison of materials to be employed for their visible fluorescence, the selection and evaluation of this system is reported.

EXPERIMENTAL

Apparatus—The Aminco-Bowman spectrophotofluorometer No. 4-8106, equipped with an Electro Instruments X-Y recorder No. 1620-809, and a detection system consisting of a Kodak Wratten filter No. 106 and a RCA 1P28 phototube were employed in this procedure.

Spectral Response of the Detection System—In order to describe the spectral response of a 106 filter–1P28 photomultiplier detection system, the following comparison of experimental to reference corrected spectra was employed. Relative quantum intensity data were obtained from a corrected fluorescent spectrum of quinine sulfate[1] by dividing the intensity at 10-mμ increments by the maximum intensity. The relative quantum intensity for the aluminum chelate of an azonaphthalene sulfonic acid dye (aluminum-PBBR chelate) has been described by White (4) and served as the second reference corrected spectrum.

Solutions of quinine sulfate (10×10^{-6} Gm./ml. in 0.1 N H_2SO_4) and the aluminum-PBBR chelate were placed in the fluorometer equipped with the 106 filter and their fluorescence emission spectra were taken in the usual manner at the appropriate excitation wavelengths (365 and 540 mμ, respectively).

Based upon these experimental spectra, values at 450 mμ for quinine sulfate and 630 mμ for aluminum-PBBR chelate were each set equal to 100% quantum intensity and values at the other wavelengths for each compound were made relative to it. Dividing these percentages by the relative quantum intensity from the corrected spectra gave quantum values which were then divided by their respective wavelengths to give energy per wavelength data. The energy data were plotted so that the values for both compounds were made equal at 580 mμ (a common wavelength nearest to that of 560 mμ, the peak for the average human eye). Values at the other wavelengths for both compounds were made relative to 100% for the maximum at 575 mμ of the combined curves. (See Fig. 1.)

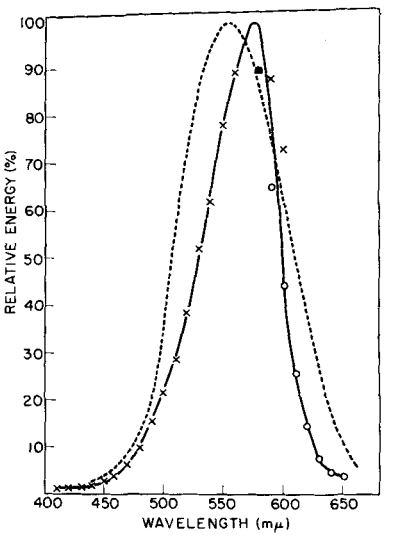

Fig. 1—Relative energy response of the 106 filter-1P28 photomultiplier system as compared to the average human eye. Key: ------, human eye responses; ——, 106-1P28 response; X, quinine sulfate data; O, Al-PBBR data.

Received June 15, 1967, from the College of Pharmacy, University of Michigan, Ann Arbor, MI 48104
Accepted for publication October 6, 1967.
This investigation was supported in part by research grant AI 05817 and training grant GM 1367 from the National Institutes of Health, U. S. Public Health Service, Bethesda, Md.
The assistance and interest of Dr. R. E. Phillips, G. K. Turner Associates, in this research is acknowledged and appreciated.
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[1] Spectrum supplied by Dr. R. E. Phillips, G. K. Turner Associates, Palo Alto, Calif.