99. Muscarine and Muscarine Isomers in Selected *Inocybe* Species[1])

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(1. V. 70)

Summary. The distribution and relative concentrations of muscarine and its isomers in *Inocybe* species were determined. Notable was the finding that *I. cinnamomea A. H. Smith*, *I. geophylla Karst.* and *I. lacera (Fr.) Quél.* contained concentrations of *epi*-muscarine equal to or higher than muscarine itself.

The reasons for a re-examination of the occurrence of muscarine in mushrooms were dicussed in papers No. 28 [2] and No. 29 [3] of this series. After the isolation of muscarine from *Amanita muscaria (L. ex Fr.) Hooker* and certain *Inocybe* species, the mother liquors were examined for the presence of the stereoisomers of muscarine. The presence of *allo*-muscarine, *epi*-muscarine, and possibly *epiallo*-muscarine, in *A. muscaria* and certain *Inocybe* species was reported [4]. Later, (–)-*allo*-muscarine was isolated from *A. muscaria* [1]. During these preliminary investigations, it was suspected that the muscarine fractions of certain *Inocybe* species contained a higher proportion of the stereoisomers of muscarine than muscarine itself. This observation provided the impetus for the present study.

Muscarine is a characteristic component of the genus *Inocybe* [5] [6] [7] [8] [9], although it is known to occur in *Clitocybe* species [10] [11]. In addition, on the basis of biological data, it has been reported to occur in several other genera of mushrooms (see review [12]). Since it has been established that the stereoisomers of muscarine are naturally occurring compounds in *A. muscaria* and probably in certain *Inocybe* species [1] [4], we undertook a systematic examination of selected *Inocybe* species, where muscarine is widely distributed, and where it often occurs in relatively high concentrations, to determine to what extent the stereoisomers of this compound are distributed throughout this genus. It is felt that these data might be useful in resolving some of the inconsistencies associated with the pharmacology and biosynthesis of muscarine, and possibly contribute to the taxonomic characters of the genus *Inocybe*.

The species selected are somewhat representative of the genus *Inocybe* and, except for *I. subbrunnea* and *I. 3398*, all are known to contain muscarine. The total muscarine fraction from each species was obtained by the method described subsequently. Partially purified extracts of muscarine chloride were pyrolysed, and the resulting norbases were separated, identified, and assayed according to the procedures of *Eugster & Schleusener* [4]. The results are summarized in the Table.

The following conclusions are noteworthy. *Nor*-muscarine and *norepi*-muscarine were identified with certainty in all species examined. *Norallo*-muscarine was detected

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Gas Chromatographic Separation of Muscarine and its Isomers as Nor-*bases*

	Inocybe species[a])	wt. in g[b])	Percent *Nor*-bases[c])			
			Muscarine	*Epi*	*Allo*	*Epiallo*
1.	*I. geophylla Karst.*	3.0	54.2	45.6	±	±
2.	*I. lilacina (Boud.) Kauff.*	15.5	99.1	0.08	0.7	±
3.	*I. pudica Kühn.*	22.5	97.6	0.4	1.6	±
4.	*I. cinnamomea A. H. Smith*	16.5	41.8	58.1	–	–
5.	*I. sororia Kauff.*	3.5	89.4	10.1	±	–
6.	*I. 3398*[d])	17.9	63.9	28.0	6.3	3.1
7.	*I. lacera (Fr.) Quél.*	6.0	46.7	53.1	±	±
8.	*I. napipes Lange*	1.3	93.0	6.9	±	±
9.	*I. fastigiata (Schaeff. ex Fr.) Quél.*	409.0	99.3	0.05	0.5	–
10.	*I. subbrunnea Kühn.*	182.0	99.0	0.1	0.7	–
11.	*I. species*[e])	800.0	99.1	0.02	0.5	0.1

[a]) Species 1–6 from Friday Harbor, Washington, USA; 7–8 from Tenino, Washington, USA; 9–11 from the vicinity of Zürich, Switzerland.

[b]) Species 1–8, dried weight; 9–11, fresh weight.

[c]) ± = results were equivocal, sum of the muscarine isomers taken as 100%.

[d]) Species not yet described in the literature. Identified by a University of Washington herbarium number assigned by *D. E. Stuntz.*

[e]) This collection was originially believed to be *I. rimosa* but later identified as a mixed collection including *I. tabacina, I. friesii, I. tarda* and *I. maculata.*

in most species whereas *norepiallo*-muscarine was identified with certainty only occasionally. It appears, therefore, that muscarine is the predominant compound in these species. However, our prime interest was directed to those species, especially, *I. lacera, I. geophylla* and *I. cinnamomea*, where a relatively high concentration of *epi*-muscarine occurs.

The significance of the latter findings cannot be assessed fully at this time, but certain points relevant to these data are worth considering. For instance, comparisons of the muscarine content of *Inocybe* species as determined by biological means [7] [8] *versus* chromatographic analyses [6] are, for the most part, in general agreement. However, with regard to *I. lacera, I. cinnamomea* and *I. napipes*, biological assays [7] [8] consistently indicate a higher concentration of muscarine than do chromatographic assays [6]. Since it is known that the isomers of muscarine differ in their pharmacologic potency [13], muscarine being considerably more active than *epi*-muscarine, we attempted to determine if the relative concentrations of muscarine isomers could explain some of the inconsistencies of different assay procedures. Curiously, the relationships are conflicting, since *I. lacera* and *I. cinnamomea* contain more *epi*-muscarine. On the other hand, our results tend to lend support to the speculations of *Malone, et al.* [8] that additional substances possessing muscarinic activity or synergistic capabilities might be present in these fungi.

The biological origin of muscarine is not known although it has been predicted that a hexose is probably the direct precursor [14] [15]. The only actual study reported to date concluded that D-glucose is not a direct precursor to the formation of muscarine in carpophores of *I. napipes* [16]. In the light of our findings, particularly those cases

where *epi*-muscarine occurs in concentrations higher than muscarine itself, suspected precursors to muscarine formation must be able to accommodate the stereospecific configuration of the existing isomers. Although there is evidence that precludes the possibility that the stereoisomers of muscarine are artefacts generated in process [1] [4], a definite conclusion with regard to the biosynthesis of muscarine must await the actual isolation of optically active *epi*-muscarine from an appropriate natural source and by using very mild extraction procedures.

Serious taxonomic considerations cannot be attempted on the basis of results derived from relatively few species, although those selected are representative of the genus *Inocybe* according to the classification system of *D.E.Stuntz*. These preliminary data tend to indicate that there is no meaningful correlation between taxonomic position and the distribution and relative concentrations of muscarine and its isomers. *Brown, et al.* [6] arrived at the same conclusion with regard to muscarine content, although the presence or absence of muscarine is considered a major character in the chemotaxonomic delineation of certain *Inocybe* species [9].

Experimental. – Mushrooms were homogenized in a Turmix blendor with 95% ethanol. The slurry was filtered with suction. The filtrate was saved and the marc was processed again in a similar manner, after which time the marc was refluxed with ethanol for two hours. The mixture was suction filtered, and all the filtrates were combined and concentrated to a lesser volume in a flash evaporator at 40°C under reduced pressure. The resulting aqeous ethanolic solution was defatted by shaking out with petroleum ether (30–60°). The defatted solution was taken to dryness and redissolved in a minimum amount of ethanol. The total quaternary ammonium compounds were precipitated as the reineckate salts and, subsequently, obtained as the total quaternary ammonium chloride fraction according to the method of *Kapfhammer & Bischoff* [17]. The muscarine chloride fraction was separated by the column chromatographic system described by *Swenberg, et al.* [11], but modified. After elution with benzene-ethanol (2:1) had indicated no muscarine when monitored by paper electrophoresis[3]), the column was washed with methanol. This procedure removed additional muscarine as well as choline. However, since choline does not interfer with subsequent analyses, the procedure was deemed satisfactory to remove as much muscarine as possible. The muscarine/choline fractions were combined, taken to dryness and stored for subsequent analyses.

The presence of muscarine and its isomers, as the *nor*-bases, was determined after pyrolysis and gas chromatographic analyses according to the procedures of *Eugster & Schleusener* [4].

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100. Synthèse de quelques dérivés de l'amino-3-didésoxy-3,5-D-lyxose

Communication préliminaire[1])

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(2 V 70)

Summary. LAH reduction of 3,5-dideoxy-1,2-O-isopropylidene-β-D-*threo*-pentofuranose-3-ulose oxime leads with a high stereoselectivity to 3-amino-3,5-dideoxy-1,2-O-isopropylidene-β-D-lyxofuranose. Some derivatives of the latter compound are described.

Dans le cadre de recherches sur la synthèse de nouveaux aminonucléosides, nous avons été amenés à préparer l'acétonide et quelques autres dérivés d'un nouvel aminosucre, l'amino-3-didésoxy-3,5-D-lyxose.

La technique utilisée [1] qui fait intervenir la réduction par l'aluminohydrure de lithium de l'oxime adéquate, s'est avérée ces dernières années offrir une excellente voie d'accès à de nombreuses osamines [2].

L'oxime **1** dont l'énantiomère L à été décrit par *Dyer et al.* [3] a été facilement obtenue. La présence presque exclusive (> 95%) de l'un des deux isomères géométriques ressort du spectre de RMN.[2]) ($(CD_3)_2CO$): $\tau = -0{,}49$, *s*, 1 p. (OH); $\tau = 4{,}17$, *d*, 1 p., $J_{1,2} = 4$ Hz (H–C1); $\tau = 5{,}13$, *d*, 1 p. (H–C2); $\tau = 5{,}13$, *q*, 1 p., $J_{4,5} = 6{,}7$ Hz (H–C4); $\tau = 8{,}48$ et 8,68, 2 *s*, 2 × 3 p. (isopropylidène); $\tau = 8{,}51$, *d*, 3 p. (H_3–C5).

L'acétylation (Ac_2O, C_5H_5N) de **1** conduit à un mélange des isomères géométriques de **2**[3]) dont les propriétés sont les suivantes: F. 92–103°. IR.: 5,67 μ (ν C=O), 7,28 et 7,34 μ (isopropylidène). RMN. *syn*-C2 (≃ 30%): $\tau = 4{,}13$, *d*, 1 p., $J_{1,2} = 4$ Hz (H–C1);

[1]) Une communication plus détaillée est destinée à paraître dans cette revue.
[2]) Sauf indications contraires 60 MHz, solvant $CDCl_3$, p. = proton, *s* = singulet, *d* = doublet, *t* = triplet, *q* = quadruplet, *qi* = quintuplet, *m* = multiplet. Interprétations du premier ordre. Attributions généralement confirmées par double résonance.
[3]) Les analyses élémentaires de tous les nouveaux produits décrits sont satisfaisantes. Elles ont été realisées par le Dr *K. Eder* (Université de Genève).