cilloylimidazole. This mechanism may also be involved in intramolecular imidazole-catalysed penicilloylations (Yamana & others, 1975).

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LETTERS TO THE EDITOR

The enolization of 4, 5-dehydromuscarone

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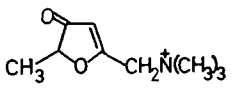
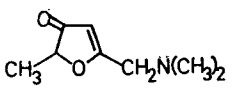
It has been stated that 4,5-dehydromuscarone (I), 4,5-dehydronormuscarone (II) and similar 3(2H)-furanones show no tendency to enolize (Rosenkranz, Allner & others, 1963; Bollinger & Eugster, 1971). However, we have found that 4,5-dehydromuscarone iodide (I) (m.p. 137–8°, prepared by the method of Meister, 1967) in aqueous (D_2O) solution at 40°, undergoes spontaneous enolization at an appreciable rate in solutions of pH greater than 5; this enolization could readily be followed with the aid of nmr spectra.

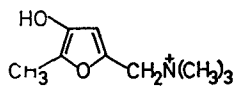


I



II

At pH 5, the proportion of the enolic form (III) present reached a maximum of 34% after 200 min and remained at this value until decomposition of the dehydromuscarone produced an acidic fragment which lowered the pH of the solution and caused a reduction in the proportion of the enolic form present (11% after 20 h). At higher pH values, enolization was rapid and more extensive.



III

Nmr spectra (Varian A60A spectrometer) of freshly prepared solutions of I showed peaks having shifts and integrals in good agreement with those expected for the keto form (δ(ppm) = 1·55, d, $-CH_3$; 3·37, s, $-N(CH_3)_3$; 4·62, s, CH_2N; 5·00, q, 2–H; 6·22, s, 4–H).

Spectra of stored solutions of I showed additional peaks with shifts and integrals (δ(ppm) = 2·26, s, CH_3; 3·12, s, $N(CH_3)_3$; 4·45, s, $-CH_2N$; 6·66, s, 4–H), in good agreement with those expected for the enolic form, the peaks for the ring methyl and proton being

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found at the lower field positions expected for a furan system. The hydroxylic proton was not observed due to exchange with deuterium. The process of enolization was accompanied by the deuteration of the proton in the 2-position, resulting in the collapse of the doublet at 1·55 δ to give a singlet, and the disappearance of the quartet at 5·00 δ. A spectrum of I in DMSO-d6 was exclusively that of the keto form, and no change occurred upon storage of the solution.

The muscarinic potencies and acetylcholinesterase inhibitory activities of solutions of 4,5-dehydromuscarone (I) incubated at pH 5 at 40° in D_2O (the conditions under which the nmr data were obtained) were determined throughout the course of an enolization. The muscarinic agonist activity was determined using guinea-pig ileum in Ringer Tyrode solution at 35° gassed with 5% carbon dioxide in oxygen in the presence of 1×10^{-4}M hexamethonium bromide. The response elicited by a constant small volume of the incubate, throughout 2¼ h of enolization, was compared directly with the responses obtained from constant concentrations of acetylcholine. The acetylcholinesterase activities were determined by a potentiometric pH-stat technique using bovine erythrocyte enzyme (Sigma Chemical Co.). Results show that, while a fall in the proportion of the ketonic form present is accompanied by a reduction in muscarinic potency in a manner which would suggest that the enolic form has negligible agonist activity, the acetylcholinesterase activity is almost unaffected.

The enolization of 4,5-dehydromuscarone (I) in aqueous solution at physiological pH value supports the view of Beckett (1967) that the equivalence of muscarinic action shown by L-(+)-muscarone, D-(—)-muscarone, allomuscarone and 4,5-dehydromuscarone *can* arise from enolization, contrary to the opinion of Bollinger & Eugster (1971). The present results also indicate that it is the keto- which is the active form at muscarinic receptors and that enolization can occur before the drug reaches these receptors. That the ketonic oxygen may be of greater importance at muscarinic sites than at acetylcholinesterase sites, where it is the ring oxygen which appears to assume a greater importance, supports the findings of recent work on cyclopentane analogues of muscarine (Sundelin, Wiley & others, 1973; Gualtieri, Giannella & others, 1974; Givens & Rademacher, 1974; Melchiorre, Gualtieri & others, 1975, a,b).

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Quantitative investigation of ergot growing in Argentina*

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Claviceps purpurea Tullasne (Hypocreaceae) grows in Argentina infecting *Cortaderia dioica Spartina alterniflora* (*S. maritima*) and other Gramineae (Ringuelet, 1936). In a previous communication Houssay & Hug (1918), reported the pharmacological activity or ergot infecting *C. dioica*, and Izquierdo & Liceaga (1947) reported the total alkaloid content of defatted ergot from *S. maritima*, as about 1% using the method of Allport & Jones (1941) and its biological assay Izquierdo (1948).

We now report the qualitative and quantitative analysis of samples of ergot collected in Samborombón Bay, Province of Buenos Aires, Argentina.

Extraction procedure and spectrophotometric determination have been described by Smith (1930) and Alexander & Banes (1961). Both methods with suitable modifications were used.

Extraction procedure. 5 g of powdered material was

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