Notes

Chem. Pharm. Bull.
11 (8) 1065 ~ 1067

UDC 547.94.07 : 582.572.4

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Chromic Acid Oxidation of Tazettine.

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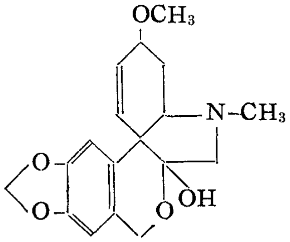
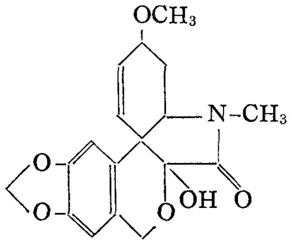
In the course of the structural studies of tazettine (I), an alkaloid frequently occurring in many species of the *Amaryllidaceae*, several oxidations have been attempted.[1~3] However, there has been recorded no successful oxidation reaction which converted its pyrrolidine moiety into the corresponding pyrrolidinone which may readily be detected by the infrared spectrum, and provide a confirmative evidence for the ring system of the molecule. Since the early days of our investigations on this alkaloid, it was known for us that the chromic acid oxidation of the base in cold acetic acid had afforded a low yield of a compound, m.p. 197°, whose structural formula had remained to be elucidated.

In the present work, increase of the yield of the product up to 40% has been realized by carrying out the oxidation under the carefully controlled conditions and enabled us to prove rigorously that the compound has a pyrrolidinone ring as anticipated.

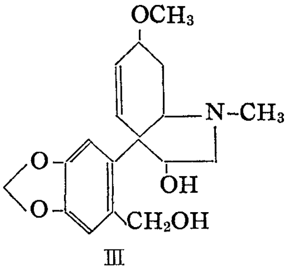
A carbonyl amide type structure was first proposed for this compound mainly because of its neutrality, since the compound was not soluble in alkali and in acid. The N-CHO structure, a possible oxidative formula of N-CH_3, was not chemically acceptable, the starting material being recovered unchanged after heating with 5% methanolic sodium hydroxide. It was then strongly suggested that this has a pyrrolidinone structure. In fact, it exhibits in the infrared spectrum an absorption band at 1715 cm^{-1} which seems to be enough to interpret as the spectrum corresponding to the



I



II



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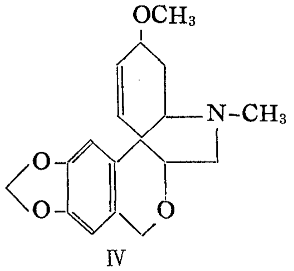

IV

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5-membered ring lactam. Confirming this view, the nuclear magnetic resonance spectrum of this compound exhibited the N-CH_3 singlet (3*H*) at $\tau=7.08$ in $CDCl_3$. The fact that no other change in the molecule of tazettine except for the moiety around N had occurred during the course of this oxidation was confirmed by its lithium alminum hydride reduction which gave tazettadiol (III),$^{1)}$ identical with the product of lithium aluminum hydride reduction of tazettine. Further confirmation was made by converting III with dilute acid into deoxytazettine (IV),$^{1)}$ identical in all respects with an authentic sample.

The above evidence established conclusively the structure (II) of this compound. This product was named oxotazettine, whose molecular formula $C_{18}H_{19}O_6N\cdot\frac{1}{2}H_2O$ agreed with the results of the elemental analysis, though the water of crystallization could not be removed by heating at 130° *in vacuo.*

Like tazettine, oxotazettine did not react with carbonyl reagents such as hydroxylamine and *p*-nitrophenylhydrazine, though it has a hemiketal moiety. Furthermore, the compound unexpectedly remained intact after attempted acetylation and *p*-nitrobenzoylation.

Experimental

Oxotazettine (II)——Chromic acid (0.5 g.) in AcOH (17 ml.) and H_2O (1 ml.) was added during 1 hr. at 10° to a stirred solution of tazettine (1 g.) in AcOH (17 ml.). The temperature was raised to 27° and the mixture stirred for 6 hr. After the solution was kept overnight at room temperature, EtOH (10 ml.) was added and the solvent was evaporated to dryness under reduced pressure. The brown oily residue was dissolved in 3% HCl (70 ml.) and the solution was extracted with Et_2O. Removal of the Et_2O gave a yellow oil (304 mg.) which on chromatography in $CHCl_3$ over activated alumina furnished an oil (300 mg.), which crystallized on trituration with EtOH. Recrystallization from 70% EtOH gave oxotazettine as needles, m.p. 196~197°. *Anal.* Calcd. for $C_{18}H_{19}O_6N\cdot\frac{1}{2}H_2O$: C, 61.01; H, 5.69; N, 3.95; OCH_3, 8.75. Found : C, 60.63; H, 5.62; N, 4.00; OCH_3, 8.97. IR : ν_{max}^{Nujol} 1715 cm^{-1}(C=O). UV λ_{max}^{EtOH} mμ (log ε) : 240 (3.70), 292 (3.66). $[\alpha]_D^{15}$ +252.6°(c=1.052, $CHCl_3$).

After basifying the acid solution and extracting with $CHCl_3$, tazettine (487 mg.), melting point and mixed m.p. 206~208°, was recovered.

Tazettadiol (III)——$LiAlH_4$ (100 mg.) was added in portions with stirring to the compound (II) (200 mg.) in dry tetrahydrofuran (10 ml.). The solution was refluxed for 4 hr. and was kept overnight at room temperature. After the addition of a few drops of H_2O, the inorganic salt was filtered off and washed with $CHCl_3$. The filtrate and washings were combined and evaporated to dryness. The residue was taken up in 3% HCl (20 ml.) which was washed with Et_2O, basified with Na_2CO_3, and extracted with $CHCl_3$. The $CHCl_3$ solution was washed with H_2O, dried and evaporated to dryness. Crystallization of the residue from Me_2CO gave tazettadiol as prisms, melting point and mixed m.p. 94~96°. The IR spectrum was identical with that of an authentic sample. *Anal.* Calcd. for $C_{18}H_{23}O_5N\cdot\frac{1}{2}H_2O$: C, 63.15; H, 7.07; N, 4.09. Found : C, 63.31; H, 7.21; N, 4.16.

Deoxytazettine (IV)——The compound (III) (80 mg.) was heated with 3% H_2SO_4 (10 ml.) on a water bath for 1 hr. After cooling, the solution was basified with Na_2CO_3 and extracted with ether. Evaporation of the ether afforded crystals (39 mg.) which on recrystallization from Et_2O gave prisms, m.p. 134~136°. The melting point was undepressed on admixture with deoxytazettine and the IR spectra were also identical.

Action of 5% Methanolic Sodium Hydroxide on the Compound (II)——The compound (II) (80 mg.) was heated with 5% methanolic NaOH (10 ml.) on a water bath for 1 hr. After cooling, the solution was saturated with NH_4Cl and extracted with $CHCl_3$. After evaporation of the solvent, the residue

Table I.

	Tazettine (mg.)	CrO_3	Reaction temperature (°C)	Crude oxotazettine (mg.)	Tazettine recovered (mg.)
1	300	100	20	62	210
2	300	200	20	74	115
3	300	150	20	86	182
4	300	150	15	57	206
5	300	150	25	90	169
6	300	150	27	91	150
7	300	150	32	65	113

was taken up in 3% HCl (10 ml.) and extracted with Et_2O, which was washed with H_2O, dried and evaporated to dryness to furnish a crystalline mass. It was recrystalized from benzene as needles, m.p. 194~196°. Mixed melting point with the starting material (II) showed no depression. The IR spectra were also identical.

The Oxidation Conditions of Tazettine with CrO_3——The yield of the compound (II) in the CrO_3 oxidation of tazettine was examined with respect to the amount of CrO_3 and to the reaction temperature, as shown in Table I.

Summary

Tazettine was oxidized with chromic acid under the controlled conditions to give a neutral compound, oxotazettine, $C_{18}H_{19}O_6N \cdot \frac{1}{2}H_2O$, which was shown to be represented by the formula (II).

(Received February 8, 1963)

[Chem. Pharm. Bull. 11 (8) 1067 ~ 1068]

UDC 616-006.6 : 612.014.46

Hideo Sugimoto : Determination of Fragility of Membrane of Tumor Cell in Saline Solution.

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It was reported by Sakurai, *et al.*[1] in 1962 that Yoshida sarcoma cells, grown in the peritoneal cavity of a rat, acquired resistance to nitrogen mustard (HN_2) 2.5 to 5 times that of the original tumor, if the inoculum cells were contacted *in vitro* with the solution of HN_2 at a concentration of 2.5×10^{-4} mM at 37° for 30 minutes immediately before inoculation. A repeated application of this procedure induced a resistance as high as 10000 times that of the original Yoshida sarcoma. These resistance indices had been determined by comparing 50% growth-inhibition concentration of HN_2 in tissue culture of the resistant and original tumor lines by the method reported by Moriwaki.[2]

Investigations on the difference in the nature of two lines, sensitive and resistant, are now being carried out from various aspects, and a result of preliminary experiment on the fragility of cell membrane in heterotonic saline solution is herein described.

For this purpose, a method for determination of fragility of platelets reported by Morita[3] was applied, in which the tumor cells (5×10^5) were suspended in 5 ml. of sodium chloride solution, ranging stepwise in concentrations from 0.9 to 0.1%. After each suspension was incubated at 37.5° for 2 hours, it was centrifuged and the supernatant was assayed for its acid phosphatase activity with *p*-nitrophenylphosphate as a substrate by the method of Ohmori[4] and Fujita.[5]

The disrupted cells release the enzyme into the medium and therefore its activity in the supernatant denotes a grade of fragility of cell membrane.

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