the walls of capillaries prior to penetration. Further, if r is small enough as expected especially for powder beds made of small particles, air expulsion from the void will be prevented in the penetration process by liquid plugs produced by capillary condensation[15] of the lower alcohols on the capillary wall, and the rate of penetration will be slower. The other alcohols will not be adsorbed onto the capillary wall so much as methyl and ethyl alcohols. The presence or absence of such adsorbed layers and the degree of absorption on the capillary wall will affect the rate of penetration and $r \cdot \cos\theta$.[16]

Then, it is necessity to see if there is a relation between $r \cdot \cos\theta$ and surface characteristics of powder particles. However, this will not discussed here.

Finally, it is concluded that the modified penetration rate method is fast, independent of operator judgment, and can be used as a simple technique to evaluate the wetting properties of powders.

Acknowledgements The authors are grateful to Professor T. Kondo of the Faculty of Pharmaceutical Sciences, Science University of Tokyo, for his valuable advices and encouragement, and Dr. S. Yamagiwa of Kyowa Chemical Industry Co., Ltd., for his continuing interest and suggestions in this work.

The authors also thank Mr. K. Tanaka for his experimental assistance in this work.

15) H. Matsumaru, *Yakugaku Zasshi*, **78**, 1205 (1958).
16) R.J. Good, *J. Colloid Interface Sci.*, **42**, 475 (1973).

[Chem. Pharm. Bull. 24(2) 336—338 (1976)]

UDC 547.854.4'551.04 : 547.866.04

Isolation of Pretazettine from *Narcissus tazetta* L.

Eiich Furusawa, Shinobu Furusawa, Shohei Tani,[1a] Hiroshi Irie, Keisuke Kitamura,[1b] and William C. Wildman[1c]

Department of Pharmacology, School of Medicine, University of Hawaii,[1a] Faculty of Pharmaceutical Sciences, Kyoto University,[1b] and Department of Chemistry, Iowa State University[1c]

(Received May 24, 1975)

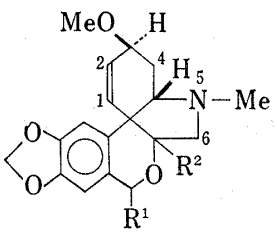
Reinvestigation of the alkaloidal constituents of *Narcissus tazetta* L. revealed that the main alkaloid was pretazettine, although the main alkaloid of this plant had been reported to be tazettine and also provided a convenient and efficient scheme for isolation of pretazettine.

In 1967, one of us (W.C.W.) reported isolation of a novel alkaloid pretazettine (I) from *Sprekelia formosissima* L. and suggested that tazettine (II) which was one of the abundant alkaloids of the Amaryllidaceae was an artifact produced by a rearrangement of pretazettine during the course of usual isolation procedure for alkaloids.[2] Based on the above finding and antileukemic activity[3] of the alkaloidal extract of *Narcissus tazetta* L., we carried out a reinvestigation of the constituents of this plant, the main alkaloid of which was reported to be tazettine.[4]

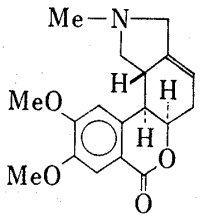
1) Location: *a) 3675 Kilauea Ave. Honolulu, Hawaii 96816*; *b) Yoshida, Sakyo-ku, Kyoto*; *c) Ames, Iowa 50010.*
2) W.C. Wildman and D.T. Bailey, *J. Am. Chem. Soc.*, **89**, 5514 (1967); *idem*, *J. Org. Chem.*, **33**, 3749 (1968).
3) E. Furusawa, N. Suzuki, S. Tani, S. Furusawa, G.Y. Ishioka, and J. Motobu, *Proc. Soc. Exp. Biol. and Med.*, **143**, 33 (1973).
4) E. Späth and L. Kahovec, *Ber.*, **67**, 1501 (1934).

Here we describe the isolation of pretazettine and other alkaloids from *N. tazetta* L. and confirm that tazettine is not a plant constituent. Pretazettine is very labile under basic conditions and column chromatography with alumina can not be used for separation and purification of the alkaloid.[2] Recently, we found that pretazettine picrate is sufficiently insoluble in dilute hydrochloric acid to provide a rapid and efficient scheme for the separation and purification of the alkaloid.

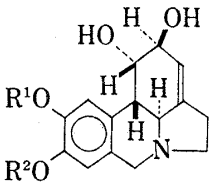
Ethanol extraction of the bulbs of *N. tazetta* and concentration provided a fraction that was soluble in dilute hydrochloric acid. Washing the aqueous, acidic solution with chloroform transferred the neutral material and homolycorine (III) to the organic layer.[5] Careful basification of the aqueous solution with ammonium hydroxide and extraction with chloroform provided the sparingly soluble lycorine (IV) and pseudolycorine (V) (identified by comparison with authentic samples) by direct filtration. The chloroform filtrate contained pretazettine as the major alkaloid (by thin-layer chromatography (TLC)). The extract was washed with dilute hydrochloric acid. Treatment of the washings with picric acid in water gave pretazettine picrate. The picrate was transformed into pretazettine hydrochloride by suspending the picrate in 3% hydrochloric acid and washing with ether several times. The aqueous layer was evaporated to dryness to give pretazettine hydrochloride which was identical in all respects with the sample previously obtained from *S. formosissima*.



I : R^1=OH, R^2=H(β)
II : R^1=H, R^2=OH(α)



III



IV : R^1, R^2=$-CH_2-$
V : R^1=H, R^2=Me

Experimental[6]

Isolation of the Alkaloids——Dormant bulbs (10 kg) were cut into small pieces and extracted with 30 liters of 95% ethanol several times under warming. The ethanol extracts were evaporated under reduced pressure and the residual gum was suspended in one liter of 3% hydrochloric acid. The suspended solution was extracted with chloroform. The aqueous layer was filtered and the filtrate was basified with aqueous ammonia under cooling with ice-salt bath and extracted with 4 liters of chloroform. The chloroform insoluble alkaloids, lycorine and pseudolycorine, deposited between two layers. Both alkaloids were collected by filtration, then separated by washing with aqueous sodium hydroxide, since pseudolycorine is a phenolic base. The chloroform extract mentioned above was concentrated to about 250 ml and set aside in a refrigerator overnight. The precipitate (remaining lycorine and pseudolycorine) was filtered again. The chloroform filtrate was washed with 0.2% hydrochloric acid (*ca.* 150 ml). A saturated solution of picric acid in water was added to the washing. The resulting yellow precipitate was collected and recrystallized from acetone to give pretazettine picrate mp 218—225° (decomp.) (*ca.* 10 g). *Anal.* Calcd. for $C_{18}H_{21}O_5NC_6H_3O_7N_3$: C, 51.43; H, 4.23; N, 10.00. Found: C, 51.13; H, 4.61; N, 9.85.

Pretazettine Hydrochloride——A suspended solution of pretazettine picrate (0.5 g) in 3% hydrochloric acid (100 ml) and ether (100 ml) was stirred several hr. The upper layer was separated and fresh ether (100 ml) was added to the aqueous solution. This procedure was repeated until the yellow color of the mixture has disappeared, The aqueous layer was evaporated to dryness under reduced pressure to give pretazettine

5) Amaryllidaceae alkaloids not containing free –OH or –NH groups usually form chloroform-soluble hydrochlorides.

6) Melting points were determined with a Yanagimoto microscope hot-stage apparatus and were uncorrected. The proton magnetic spectrum and INDOR spectra for determination of coupling constants of each protons were measured in $CDCl_3$ (tetramethylsilane as internal standard) using a Varian HA-100D Spectrometer which was modified for INDOR experiments.

hydrochloride (0.25 g) mp 234—236° (from EtOH)[7] which was identical with pretazettine hydrochloride isolated previously from *S. formosissima* in mixed mp, infrared spectrum (Nujol) and TLC (silica gel) in several different solvent systems. Pretazettine, regenerated from its hydrochloride by treatment with aqueous ammonia under cooling, was a glass and exhibited M^+ 331 in its mass spectrum and the following signals in its proton magnetic resonance spectrum: δ ($CDCl_3$); 6.83 and 6.75 (two aromatic protons s.) 6.05 (C_8–H, s.), 5.86 (methylenedioxy 2H, s.), 5.81 (C_2–H, broad d., $J=11.0$ Hz.), 5.48 (C_1–H, t. of d, $J=1.8$ and 11.0 Hz., long range coupling with C_3–H and C_{4a}–H by four sigma W-arrangement), 4.31 (C_{6a}–H, d. of d., $J=7.7$ and 11.0 Hz.), 4.10 (C_3–H, m.), 3.41 (OMe, s.), 2.97 (C_6–H_α, d. of d., $J=9.9$ and 11.0 Hz.), 2.63 (C_6–H_β, d. of d., $J=9.9$ and 7.7 Hz.), 2.86 (C_{4a}–H, m.), 2.48 (N–Me, s.), and 1.77 (C_4–H_β, diffused t.).

Acknowledgement This work was supported in part by Research Grant from National Cancer Institute (CA 12733) and from American Cancer Society (IC-29).

7) Melting point of newly isolated pretazettine hydrochloride was slightly higher than that of the previous one; it is thought to be due to the apparatus used.

[Chem. Pharm. Bull. 24(2) 338—341 (1976)]

UDC 547.854.4'551.04 : 547.866.04

New Syntheses of Alloxazine 5-Oxides and Fervenulin 4-Oxides by the Nitrative Cyclization

Yoshiharu Sakuma, Shigeru Matsumoto, Tomohisa Nagamatsu,[1] and Fumio Yoneda[1a]

Faculty of Pharmaceutical Sciences, Kumamoto University[1]

(Received May 29, 1975)

The treatment of 6-anilinouracils with potassium nitrate in acetic acid in the presence of sulfuric acid led to the exclusive formation of the corresponding alloxazine 5-oxides. Similarly, the treatment of 6-benzylidenehydrazino-1,3-dimethyluracils with the same reagents gave the corresponding fervenulin 4-oxides.

The aromatic nitro group undergoes intramolecular dehydrative cyclization with a substituent in the molecule possessing active hydrogen to form a heterocyclic N-oxide. A number of this type of reactions were briefly surveyed in the textbooks by Ochiai[2] and by Katritzky and Lagowski,[3] and furthermore covered more extensively by Preston and Tennant.[4]

We have now found that the nitration of 6-anilinouracils, does not lead to the 5-nitro derivatives, but exclusively to alloxazine 5-oxides. The reaction is equally applicable to 6-benzylidenehydrazino-1,3-dimethyluracils to give fervenulin 4-oxides. This paper describes a detailed account of this new convenient method for the syntheses of alloxazine 5-oxides and fervenulin 4-oxides, which was mentioned in part in our previous communication.[5]

A mixture of 6-anilinouracils (Ia—o)[6,7] and slight excess of potassium nitrate in acetic acid including sulfuric acid was stirred at 90° for a while, during which time the reaction mixture changed its colour from pale yellow to brown. The concentration of the solvent under reduced pressure followed by dilution with water gave exclusively the corresponding alloxazine 5-oxides (IIa—o) in good yields (Table I).

1) Location: *Oe-honmachi, Kumamoto 862, Japan*; *a*) To whom inquiries should be addressed.
2) E. Ochiai, "Aromatic Amine Oxides," Elsvier, New York, 1967, pp. 59—62.
3) A.R. Katritzky and J.M. Lagowski, "Chemistry of Heterocyclic N-Oxides," Academic Press, New York, 1971, pp. 120—141.
4) R.N. Preston and G. Tennant, *Chem. Rev.*, **72**, 627 (1972).
5) F. Yoneda and Y. Sakuma, *Chem. Pharm. Bull.* (Tokyo), **21**, 448 (1973).
6) H. Goldner, G. Dietz, and E. Carstens, *Ann.*, **694**, 142 (1966).
7) F. Yoneda, S. Matsumoto, and Y. Sakuma, *J. C. S. Perkin I*, **1975**, 1907.