Alloxan hydroperoxide is less stable than the hydrate and loses its peroxide character completely on standing for several hours in the presence of traces of moisture and after several weeks in the desiccator.

Dr. Byron Olson[7] has kindly tested alloxan hydrate (III) and alloxan hydroperoxide (II) as well as equimolar mixtures of alloxan hydrate and hydrogen peroxide in the desensitization test against tubercle bacilli (BCG) in guinea pigs.[8] The statistical evaluation of the tests involving several hundred animals is still in progress and will be reported elsewhere. There seem to be distinct differences between II and III.

The adducts IV and V of alloxan with hexyl and dodecyl alcohol,[9] prepared in the hope of obtaining lipophilic compounds suitable for diabetogenic tests, were soluble in ether and hexane but easily hydrolyzed by the addition of water.

Experimental

Anhydrous Alloxan (I).—Alloxan hydrate was sublimed at 210° (0.1 mm.) and formed lemon-yellow crystals, m.p. 253° (reported 250–255°).[2]

Anal. Calcd. for $C_4H_2N_2O_4$: C, 33.81; H, 1.42. Found: C, 33.55; H, 1.48 (the previous analysis[5] was too high in hydrogen).

Alloxan Hydroperoxide (II).—A suspension of 1 g. (7.04 mmoles) of powdered anhydrous alloxan in 100 ml. of dry ether was treated with 20 ml. (40 mmoles) of hydrogen peroxide in absolute ether.[10] After about two hours the clear colorless solution was decanted from a small amount of solid and concentrated *in vacuo* to 30 ml. The shiny, colorless crystals (1.23 g., 72%) were collected and washed with ether and pentane. On slow heating a sample melted with decomposition at 172–176° (m.p. of alloxan hydrate is 170° dec.). On a hot-stage preheated to 112° the sample evolved gas at 120°.

Anal. Calcd. for $C_4H_2N_2O_4 \cdot H_2O_2$: C, 27.27; H, 2.29; N, 15.91. Found: C, 27.12; H, 2.38; N, 15.95.

Titration for Active Oxygen.—The hydroperoxide (II, 48.8 mg.), dissolved in a mixture of 40 ml. of 0.18 *N* sulfuric acid and 10 ml. of 10% potassium iodide solution, was treated with 2 ml. of 3% molybdic acid solution. After 5 min., titration of the triiodide in solution with 5.05 ml. of 0.1 *N* sodium thiosulfate solution indicated that the sample contained 91.1% hydroperoxide. Samples stored over P_2O_5 contained 89.3% after one day, 87.4% after five days and no active oxygen after two months. Samples kept in screw cap vials outside the desiccator contained 1.76% of active oxygen after two days.

Alloxan–Hexanol Adduct (IV).—The adduct was obtained by adding 1.6 g. of anhydrous alloxan to 15 ml. of hexanol to which some dry hydrogen chloride had been added. The mixture was warmed until the solution became clear. On cooling, the mixture solidified to a waxy white mass. The excess hexanol was dissolved in pentane and the colorless, waxy flakes of the adduct were collected (2.3 g., 85% yield), m.p. 251–265° dec. The compound was soluble in ether but it could not be recrystallized satisfactorily from ether–pentane mixtures. The reaction product, therefore, was analyzed directly.

Anal. Calcd. for $C_4H_2O_2N_2 \cdot C_6H_{13}OH$: C, 49.17; H, 6.60; N, 11.47. Found: C, 48.51; H, 6.42; N, 10.32.

Alloxan–Dodecanol Adduct (V).—The adduct, prepared in a manner similar to IV, yielded shiny colorless leaflets, melting at 104–120°, readily soluble in ether and slightly soluble in hexane; the addition of water caused the separation of dodecyl alcohol in oily drops.

Anal. Calcd. for $C_4H_2O_4N_2 \cdot C_{12}H_{25}OH$: C, 58.51; H, 8.60; N, 8.53. Found: C, 60.49; H, 9.09; N, 7.04.

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(7) We are greatly indebted to Dr. Byron Olson, Laboratory of Tropical Diseases, National Microbiological Institute, for his great interest in this investigation and for kindly making arrangements for the various tests.

(8) D. A. Long, A. A. Miles and W. L. M. Perry, *Lancet*, **902**, 1085 (1951).

(9) *Cf.* H. Biltz and E. Topp, *Ber.*, **45**, 3667 (1912).

(10) See ref. 6, p. 33.

Solubilities of Some 2,3,5-Triphenyltetrazolium Salts

BY SAMUEL WEINER

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In spite of the interest in the biochemical properties of the triaryltetrazolium salts, few data are available on their physical properties. Some of the slightly soluble salts of 2,3,5-triphenyltetrazolium were studied in this Laboratory and in the course of the work solubilities were measured at 25.00°.

SOLUBILITIES OF 2,3,5-TRIPHENYLTETRAZOLIUM

Abbrev.: g, gravimetric by evaporation of equilibrated solution; i, iodometric by oxidation to iodate and titration of latter[4]; t, titration with picrate[1]; c, colorimetric; n.c., new compound.

2,3,5-Triphenyltetrazolium	Solubility at 25.00° G./liter	Moles/liter	Method	Lit. Ref.
Iodide	1.308 ± 0.004	$(3.07 \pm 0.01) \times 10^{-3}$	g,i,t	2
Picrate	1.037 ± .002	$(7.0 \pm .4) \times 10^{-5}$	g	3
Triiodide	1.0273 ± .0007	$(40 \pm 1) \times 10^{-6}$	g	2
Dichromate	1.626 ± .004	$(7.69 \pm .05) \times 10^{-4}$	g,c	n.c.
Perchlorate	1.307 ± .001	$(7.71 \pm .02) \times 10^{-4}$	g	n.c.
Thiocyanate	1.51 ± .01	$(4.22 \pm .02) \times 10^{-3}$	g	3

From measurements of the solubility of the iodide in NaCl solutions it was calculated that the ideal solubility at zero ionic strength was 2.95×10^{-3} *M*. Its mean activity coefficients are 0.93 at 0.01 molar ionic strength, 0.89 at $\mu = 0.04$, and 0.85 at $\mu = 0.09$.

Melting points not reported previously in the literature are (all corrected): 2,3,5-triphenyltetrazolium iodide 228–229° dec., perchlorate 269–270°, dichromate 218–219° (dec., with darkening a few degrees below). The picrate, reported melting at 186–188°,[3] melted in this range when first prepared, but after long standing or when well dried or recrystallized from toluene it melted at 193–194°. The thiocyanate, reported melting at 134–136°,[3] melted at 174–174.5°.

The permanganate[2] was found to explode at 135°; it is insoluble in hot water or hot benzene. The ferricyanide, not previously reported, is yellow, insoluble in organic liquids, very slightly soluble in hot water, and explodes at 228°.

The perchlorate is colorless but turns yellow if exposed to light; it is soluble in ethanol or acetone, insoluble in chloroform or CCl_4, recrystallizable from acetic acid as needles. *Anal.* (by Univ. of Wis. microchemical laboratory). calcd.: C, 57.05; H, 4.02; N, 14.00. Found: C, 57.15; H, 4.00; N, 14.19.

The dichromate was 12.8 ± 0.1% Cr (calcd. 12.78); it is lemon-yellow when freshly precipitated but orange-yellow after drying, and explodes if heated much above the melting point.

(1) S. Weiner, *Chem.-Anal.*, **42**, 9 (1953).

(2) H. von Pechmann and P. Runge, *Ber.*, **27**, 2920 (1894).

(3) D. Jerchel and H. Fischer, *Ann.*, **563**, 200 (1949).

(4) I. M. Kolthoff and E. B. Sandell, "Textbook of Quantitative Inorganic Analysis," Rev. Ed., The Macmillan, Co., New York, N. Y., pp. 628–629, modified.

A characteristic of some of these salts, especially the iodide, is their tendency to develop electrostatic charges, interfering with accurate weighing or transferring of small samples. All preparations were by simple metathesis without oxidation–reduction or change of structure.

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Isolation of Tazettine and Lycorine from Certain *Hymenocallis* Species

BY W. C. WILDMAN AND CAROL J. KAUFMAN

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As part of a systematic survey of the alkaloids of the *Amaryllidaceae* we wish to report the isolation of tazettine and lycorine from several *Hymenocallis* species. The alkaloid tazettine has been isolated from *Lycoris radiata*,[1,2] *Narcissus tazetta*,[3] *Ungernia sewerzowii*[4,5] and *Galanthus nivalis*.[6] Although Greathouse and Rigler[7] have demonstrated the presence of unspecified alkaloids in *H. galvestonensis*, the only chemical study of *Hymenocallis spp.* was conducted by Gorter[8] who isolated lycorine from *H. littoralis* in 0.0015% yield. Lycorine appears to be the most wide-spread alkaloid of the *Amaryllidaceae*.

The isolation of a crude alkaloid fraction from the bulbs was achieved by a conventional procedure. Nearly 90% of this crude fraction was soluble in benzene and concentration of the benzene solution gave most of the tazettine that could be isolated. A minute quantity of lycorine was detected in the benzene-insoluble residues.

TABLE I

ISOLATION OF TAZETTINE AND LYCORINE FROM *Hymenocallis spp.*

Species	Yield of crude alkaloids, %	Yield of tazettine, %	Yield of crude lycorine, %
H. caymanensis Herb.[a]			
3.59 kg. bulbs only	0.09[c]	0.062	0.004
20.4 kg. bulbs only	.07[c]	.045	.001
3.24 kg. leaves only	.15[d]	.009[d]	...
H. occidentalis (leConte) Kunth[b]			
3.93 kg. bulbs only	.14	.046	.004
H. littoralis Salisb.[e,f]			
240 g. bulbs only	.09	.03	...

[a] Obtained from the Los Angeles State and County Arboretum, Arcadia, California. [b] Obtained from W. H. Duncan, University of Georgia, Athens, Georgia. [c] All yields are based on wet weight of bulb unless otherwise designated. [d] Based on dry weight of leaves; loss of weight on drying at 71°, 91%. [e] Obtained from C. G. Van Tubergen, Ltd., Haarlem, The Netherlands. [f] Isolation performed by Dr. H. A. Lloyd of this Laboratory.

Tazettine and lycorine were identified by analytical data and rotation and by the preparation of known derivatives. The infrared absorption spectrum of the lycorine isolated was identical with that of an authentic sample.[9] While the structure of lycorine has been largely elucidated, only portions of the tazettine molecule are known with certainty.[10]

Experimental[11]

Isolation of the Crude Alkaloid Fraction.—The bulbs of *H. caymanensis*, 20.4 kg., were ground in a Ball and Jewell grinder. The ground material was mixed with 20 l. of ethanol containing 160 g. of *d*-tartaric acid and allowed to stand overnight. The mixture was stirred for 2 hours at 55°. The solution (4 l.) was siphoned from the solid material and the solid was stirred with 8 l. of 1% ethanolic tartaric acid for 2 hours at 55°. The mixture was allowed to stand two days at 20° and then approximately 10 l. of solution was removed through a siphon. The residue was stirred with 11 l. of 1% ethanolic tartaric acid at 55° for 2 hours. The solution was siphoned from the solid and the liquid remaining with the solid was removed by vacuum filtration. The combined solutions were filtered in an International Chemical Centrifuge and concentrated to 5 l. The concentrate was diluted with 2.5 l. of water, treated with 2 l. of 2 *N* hydrochloric acid and extracted 16 times with 200-ml. portions of chloroform. The chloroform solution was discarded after a test for alkaloids proved negative. The aqueous solution was made basic with solid potassium carbonate and extracted 45 times with 200-ml. portions of chloroform. The chloroform solution was extracted 38 times with 200-ml. portions of 2 *N* hydrochloric acid and the aqueous solution was made basic with solid potassium carbonate. The alkaloid was removed from the aqueous solution by 30 extractions with 200-ml. portions of chloroform. The chloroform solution was concentrated, dried and further concentrated to constant weight, 14.99 g. (0.0734%).

Isolation of Tazettine and Lycorine.—A portion of the crude alkaloid mixture from *H. caymanensis* weighing 1.84 g. was extracted with 25 ml. of thiophene-free benzene and the hot solvent was decanted from the gummy residue. This residue, 0.22 g., was treated in a similar manner with 10 ml. of hot ethyl acetate. The material that was insoluble in ethyl acetate, 0.14 g., was dissolved in 5 ml. of hot ethanol. The ethanolic solution crystallized upon standing to give 12 mg. of crude lycorine, m.p. 250–255° dec. Concentration of the benzene solution gave crude tazettine which was recrystallized twice from ethanol, 0.70 g., m.p. 208–210° dec. Concentration of the ethyl acetate extract and trituration with ethanol gave an additional 0.02 g. of tazettine, m.p. 202–208° dec. The mother liquors from the crystallization of tazettine were dissolved in 20 ml. of benzene and chromatographed on 31.0 g. of alumina. Elution with ethyl acetate followed by chloroform gave an additional 0.35 g. of crude tazettine which was recrystallized from ethanol, 0.23 g., m.p. 207–209° dec. An additional 50 mg. of crude lycorine, m.p. 223–232° dec., was obtained from the chloroform–ethanol eluates of this column. The total yield of crude lycorine was 62 mg. (0.004% based on the wet weight of the bulb). The total yield of tazettine based on the wet weight of the bulb was 0.062%. An analytical sample of tazettine was crystallized

(1) H. Kondo, K. Tomimura and S. Ishiwatari, *J. Pharm. Soc. Japan*, **52**, 51 (1932).

(2) E. Späth, H. Kondo and F. Kuffner, *Ber.*, **69**, 1086 (1936).

(3) E. Späth and L. Kahovec, *ibid.*, **67**, 1501 (1934).

(4) S. Norkina and A. Orechoff, *ibid.*, **69**, 500 (1936).

(5) E. Späth, A. Orechoff and F. Kuffner, *ibid.*, **69**, 2446 (1936).

(6) G. R. Clemo and D. G. I. Felton, *Chemistry and Industry*, 807 (1952).

(7) G. A. Greathouse and N. E. Rigler, *Am. J. Botany*, **28**, 702 (1941).

(8) K. Gorter, *Bull. Jard. Bot. Buitenzorg*, [3] **1**, 352 (1920).

(9) Kindly furnished by Prof. K. Wiesner.

(10) The chemistry of tazettine, lycorine and other alkaloids of the *Amaryllidaceae* has been reviewed recently by J. W. Cook and J. D. Loudon in R. H. F. Manske, "The Alkaloids," Vol. II, Academic Press, Inc., New York, N. Y., 1952, p. 331. For more recent developments in the chemistry of the two alkaloids, see E. J. Forbes, J. H. Mason and R. Robinson, *Chemistry and Industry*, 946 (1953); K. Wiesner, W. I. Taylor and S. Uyeo, *ibid.*, 46 (1954); T. R. Govindachari and B. S. Thyagarajan, *ibid.*, 374 (1954); R. Robinson, *ibid.*, 1317 (1953); E. Wenkert, *ibid.*, 1088 (1953); R. B. Kelly, W. I. Taylor and K. Wiesner, *J. Chem. Soc.*, 2094 (1953); H. Kondo and coworkers, *Annual Rept. ITSUU Lab. (Tokyo)*, **1**, 21 (1950); *ibid.*, **2**, 18 (1951), *ibid.*, **3**, 65 (1952), [*C. A.*, **47**, 7516 (1953)]. *ibid.*, **4**, 30 (1953).

(11) All melting points were observed on a Kofler microscope hot-stage and are corrected. Analyses were performed by Dr. W. C. Alford and his staff. Infrared and ultraviolet absorption spectra were determined by Mrs. Iris J. Siewers.