14. ***m*-(2,4-Diamino-s-triazinyl-6)-amino-*p*-hydroxyphenylarsonic Acid.**—A solution of 22.3 g. of 4-hydroxy-3-aminophenylarsonic acid in 200 cc. of 2% sodium hydroxide is run dropwise, with stirring, within one hour, into a fine suspension of 20 g. of cyanuryl chloride in 400 cc. of water to which 100 g. of chipped ice and 1 cc. of octyl alcohol had been added. The *p*H is adjusted to 7.4. Stirring is continued at 0 to 2° until a filtered sample does not give any color reaction with diazobenzenesulfonic acid. The reaction mixture, kept at 10°, is saturated with gaseous ammonia. The temperature is then raised slowly to the boiling point of the mixture. After most of the excess ammonia is driven off, the hot solution is bone-blacked and filtered. The cleared filtrate yields on acidification with dilute hydrochloric acid a white precipitate which is purified as in the described manner.

Anal. Calcd. for $C_9H_{12}O_4N_6As$: As, 21.89; N, 24.57. Found: As, 21.75; N, 24.48.

15. ***p*-(2,4-Diamino-s-triazinyl-6)-amino-*o*-hydroxyphenylarsonic Acid.**—Preparation in all ways as in (14) using 4-amino-2-hydroxyphenylarsonic acid.

Anal. Calcd. for $C_9H_{12}O_4N_6As$: As, 21.89; N, 24.57. Found: As, 21.84; N, 24.49.

16. ***p*-(2,4-Diamino-s-triazinyl-6)-aminophenylarsine Oxide.**—Ten grams of *p*-(2,4-diamino-s-triazinyl-6)-aminophenylarsonic acid is dissolved in 500 cc. of 10% hydrochloric acid. One gram of potassium iodide, dissolved in a small amount of water is added and the solution stirred and saturated with sulfur dioxide while the temperature is maintained at 35°. The arsine oxide precipitates slowly in the form of a white powder. After standing for twenty-four hours it is filtered off, washed with ice water and dried *in vacuo*.

The compound is soluble in dilute sodium hydroxide solution, and in the dissolved state it reduces Fehling solution. Hydrochloric or acetic acid precipitates the product from an alkaline solution.

Anal. Calcd. for $C_9H_9ON_6As$: As, 25.64; N, 28.77. Found: As, 25.72; N, 28.74.

17. ***p,p'*-Bis-(2,4-diamino-s-triazinyl-6)-aminoarsenobenzene.**—A mixture prepared from 30 g. of sodium hypophosphite, which had been dissolved in 60 cc. of 38% hydrochloric acid, 200 cc. of methanol and 0.5 cc. of 48% hydriodic acid is added, in a current of nitrogen, to 20 g. of *p*-(2,4-diamino-s-triazinyl-6)-aminophenylarsonic acid which had been dissolved in 300 cc. of 4% hydrochloric acid. During the addition, the solution of the arsonic acid is stirred and maintained at 70°. A yellow precipitate is formed which is filtered off and washed with water, methanol and ether and dried *in vacuo*. It is insoluble in water, methanol and ether. It remains unchanged when heated to 230°. It starts to discolor at higher temperatures and chars at around 250°.

Anal. Calcd. for $C_{18}H_{18}N_{12}As_2$: As, 27.13; N, 30.44. Found: As, 27.09; N, 30.37.

Summary

The introduction of the s-triazine ring into the amino group of arsanilic acid enhances the trypanocidal and spirochetocidal properties of this compound, provided that at least one unsubstituted amino group is attached to a carbon atom of the triazine ring.

The optimum effect is obtained in *p*-(2,4-diamino - s - triazinyl - 6) - aminophenylarsonic acid. The corresponding arsinoxide and arseno compound are described.

160 WEST 59TH STREET
NEW YORK 19, N. Y.

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[CONTRIBUTION FROM THE NATIONAL MEDICAL COLLEGE OF SHANGHAI]

The Constituents of Fritillaria roylei[1]

BY YUN-HSI WU

T. Q. Chao[2] isolated two alkaloids from the roots of the *Fritillaria roylei*, the source of one variety of the common Chinese drug Pei-Mu growing in Chekiang, China. Chao named the alkaloids peimine and peiminine, and K. K. Chen studied their pharmacology.[3] An extensive analytical characterization of peimine was conducted by Chi, Kao and Chang.[4] Chao,[5] on investigating Pei-Mu of the Szechuan variety, encountered an entirely different active principle, fritimine.

In the present investigation of *Fritillaria roylei*, the author has further characterized the alkaloids peimine and peiminine and has succeeded in isolating a hitherto unknown neutral, nitrogen-free product which appears to be related to the alkaloids and for which the name propeimin is suggested.

Peimine.—Chao[2] suggested for this alkaloid the formula $C_{19}H_{30}O_2N$, but the value which he found for nitrogen was over one per cent. higher than subsequently established.[4] Chi, Kao and Chang[4] reported a total of twenty-six analyses of peimine and its salts and on the basis of the results assigned the formula $C_{26}H_{43}O_3N$.

In the present investigation the yield, 1.5 g. of peimine from 50 kg. of drug, was the same as that given by Chi, Kao and Chang. Although the previous workers state that the alkaloid is inactive, the preparation showed a rotation of $[\alpha]^{18}D$ −19.2°. Peimine is a saturated alkaloid. It contains neither methylimide nor methoxyl groups. A Zerewitinoff determination indicated the presence of two active hydrogen atoms per mole. The analyses are in better accord with the formula $C_{27}H_{45}O_3N$ than with that given by Chi, Kao and Chang, as shown in the following summary

(1) The author is greatly indebted to Professor A. Butenandt, director of the Kaiser-Wilhelm Institut für Biochemie, for his valuable suggestions in carrying out the work, to Professor G. Oddo of the University of Palermo for sending a sample of solanidine-s for comparison, and to Professor L. F. Fieser of Harvard University for arranging this paper for publication.

(2) Chao, *Chinese J. Physiol.*, **6**, 265 (1932).

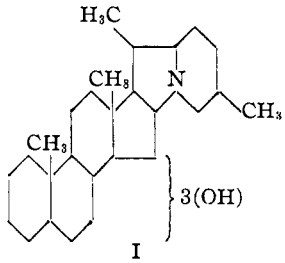
(3) Ref. 2, note by K. K. Chen.

(4) Chi, Kao and Chang, THIS JOURNAL, **58**, 1306 (1936).

(5) Chao, *Chinese J. Physiol.*, **7**, 41 (1933).

	Deviations from $C_{26}H_{43}O_3N$		Deviations from $C_{27}H_{45}O_2N$	
% C	+0.42	+0.26	+0.06	−0.10
% H	+0.13	+0.14	0.00	+0.01
% N	+0.06		+0.17	

The C_{27}-formulation suggests a possible relationship to one of the known classes of steroid alkaloids. Solanidine-s is a mono-unsaturated alcohol of the formula $C_{27}H_{43}ON$,[6,7] and, like this substance, peimine is very resistant to the classical methods of degradation. Thus peimine may be a dihydroxydihydrosolanidine (I), in which one hydroxyl group is inert to Grignard reagent. Or it may be related to the veratrine alkaloids[7]; rubijervine, the closest in composition to peimine, is a mono-unsaturated dialcohol $C_{27}H_{43}O_2N$ having the same ring system as I, and hence peimine may correspond to a hydroxydihydrorubijervine.



I

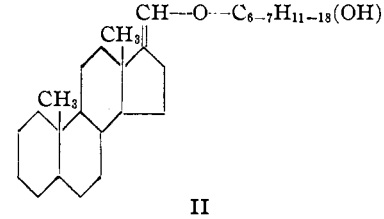
Peiminine.—This companion alkaloid was found present to the extent of 0.3 g. per 50 kg. of drug. Chao[2] proposed the formula $C_{18}H_{28}O_2N$, but again his nitrogen value was over one per cent. higher than indicated by the present analyses. Our results agree best with the formula $C_{25}H_{41}O_3N$, and hence the substance may be a lower homolog of peimine.

Propeimin.—This new neutral product is present in about the same quantity as peimine. The analyses agree closely with the calculated values for $C_{26}H_{44}O_3$, while analyses of the diacetate are in better accord with the formulation $C_{27}H_{46}O_3$ for the parent compound.

		Deviations from C_{26}-formulation	Deviations from C_{27}-formulation
Free compound	% C	−0.06	−0.34
	% H	−0.01	−0.13
Diacetate	% C	+0.26	−0.08
	% H	−0.03	−0.14

Thus a choice cannot be made at present between the C_{26}- and C_{27}-formulations. The formula $C_{27}H_{46}O_3$ would correspond to a dihydro derivative of a steroid sapogenin having a single nuclear hydroxyl group, for example sarsasapogenin or smilagenin, of the formula $C_{27}H_{44}O_3$. The substance does not exhibit color reactions characteristic of phytosterols, and it is stable to alkali. The formation of a diacetyl derivative indicates that two of the three oxygen atoms are present as hydroxyl groups. The third oxygen may be present in an enol ether grouping, for on mild treatment of propeimin with alcoholic hydrochloric acid an extensive carbon-fragment is cleaved from the molecule with the formation of a crystalline compound (m. p. 167°) of the formula $C_{20}H_{32}O$ (or possibly $C_{19}H_{30}O$ or $C_{21}H_{34}O$).

A second degradation product was obtained by the oxidation of propeimin with chromic acid at room temperature. There resulted in good yield a saturated ketone, m. p. 115.5°, of the formula $C_{19}H_{30}O$. The ketone was characterized by the preparation and analysis of an oxime (m. p. 201°), a hydrocarbon $C_{19}H_{32}$ melting at 55° (by Wolff–Kischner reduction), and a formyl derivative, which indicates the presence of an adjacent methylene group. The ketone is isomeric with androstanone (m. p. 122°), but it depresses the melting point of this substance and it shows no androgenic activity (Fussgänger test). It melts 10° higher than the isomer etiocholanone-17, and the hydrocarbon resulting from the Wolff-Kischner reduction melts some 25° lower than etiocholane and gives a depression when mixed with this substance (the comparison sample was obtained from cholestenone through the intermediates coprostanone, coprostane, and etiocholanone). The melting point of the parent hydrocarbon is not far from that of androstane (m. p. 51–52°), but a comparison has not yet been possible. A dehydrogenation experiment was attempted with 300 mg. of propeimin but no satisfactory products could be isolated. Although the nature of the carbon skeleton has not been established, the author suggests the provisional structure II to account for the known facts.



II

The substance appears particularly interesting because of the suggestions of associations to both steroid alkaloids and steroid sapogenins. A continuation of the investigation is planned.

Experimental Part

Peimine.—Fifty kilograms of coarsely powdered *Fritillaria roylei* was divided in eight parts and extracted with alcohol in a countercurrent process, the temperature being kept around 50°. The alcoholic extract was evaporated under diminished pressure and the residue was extracted repeatedly with 5% hydrochloric acid to remove the alkaloids. The acid solution was carefully made alkaline with dilute sodium carbonate and extracted many times with ether. Dry hydrogen chloride was passed into the dried ethereal solution of the alkaloids; the mixture of hydrochlorides of peimine and peiminine separated at first as white flakes, and these gradually changed to a yellowish oil adhering to the walls of the container. The mixture was

(6) Prelog and Szpilfogel, *Helv. Chim. Acta*, **25**, 1306 (1942).

(7) Craig and Jacobs, *J. Biol. Chem.*, **149**, 451 (1943); Jacobs and Craig, *ibid.*, **152**, 64 (1944).

dissolved in water and the crude alkaloids set free by neutralization with sodium carbonate. The basic material was then recrystallized from 90% alcohol until the least soluble fraction, consisting of peimine, had a constant optical rotation. The fully purified alkaloid melted at 215° and had a rotation of $[\alpha]^{18}D$ −19.2° (46.9 mg. in 2 cc. alcohol, α = −0.45°).

Anal. Calcd. for $C_{26}H_{43}O_3N$: C, 74.77; H, 10.38; N, 3.36. Calcd. for $C_{27}H_{45}O_3N$: C, 75.13; H, 10.51; N, 3.25. Found: C, 75.19, 75.03; H, 10.51, 10.52; N, 3.42.

Peiminine.—The alkaloidal material recovered from the mother liquors of the crystallization of peimine was dissolved in acetone and fractionally precipitated with water. The portions melting around 130° were collected and recrystallized from dilute alcohol and afforded 300 mg. of peiminine melting at 135°; $[\alpha]^{18}D$ −67.3° (11.6 mg. in 2 cc. alcohol, α = −0.39°). Chao[2] reported m. p. 135°, $[\alpha]_D$ −62.5°.

Anal. Calcd. for $C_{25}H_{41}O_3N$: C, 74.39; H, 10.24; N, 3.47. Found: C, 74.52; H, 9.85; N, 3.36.

Propeimin.—The alkaloid-free residue after being dried in vacuum was brought into solution by using a little alcohol and much ether. The solution was shaken with sodium hydroxide to remove acidic impurities, dried and evaporated, and the residue was treated with petroleum ether to remove oily constituents. The residual crude propeimin was crystallized alternately from benzene and alcohol to constant optical rotation. The purified substance crystallizes in either plates or needles, both melting at 186°; $[\alpha]^{18}D$ −37.8° (24.3 mg. in 2 cc. alcohol, α = −0.46°).

Anal. Calcd. for $C_{26}H_{44}O_3$: C, 77.18; H, 10.96. Calcd. for $C_{27}H_{46}O_3$: C, 77.46; H, 11.08. Found: C, 77.12; H, 10.95.

Propeimin Diacetate.—A solution of 150 mg. of propeimin in dry pyridine was treated with a few drops of acetyl chloride and allowed to stand overnight. The product which precipitated on pouring the solution into water when crystallized from alcohol melted at 140°.

Anal. Calcd. for $C_{30}H_{48}O_5$: C, 73.72; H, 9.91. Calcd. for $C_{31}H_{50}O_5$: C, 74.06; H, 10.02. Found: C, 73.98; H, 9.88.

Oxidation of Propeimin: Ketone $C_{19}H_{30}O$.—A solution of 100 mg. of propeimin in glacial acetic acid was treated with 33 mg. of chromic anhydride and allowed to stand for twenty-four hours at room temperature. The oxidation product was precipitated with water and recrystallized from alcohol; yield 60 mg., m. p. 115.5°, $[\alpha]^{18}D$ −36.3° (4.05 mg. in 0.3 cc. alcohol, α = −0.49°).

Anal. Calcd. for $C_{19}H_{30}O$: C, 83.15; H, 11.02. Found: C, 83.26; H, 10.81.

The **oxime,** prepared by refluxing 40 mg. of the ketone in alcohol with 50 mg. of hydroxylamine hydrochloride and 60 mg. of sodium acetate for two hours, was precipitated with water and recrystallized from alcohol; it melted at 201°.

Anal. Calcd. for $C_{19}H_{31}ON$: C, 78.83; H, 10.80; N, 4.84. Found: C, 78.84; H, 10.79; N, 4.95.

The **formyl derivative** was prepared by dissolving 50 mg. of the ketone $C_{19}H_{30}O$ in benzene in a 10-cc. Erlenmeyer flask and adding a few drops of ethyl formate and chips of sodium metal. The flask was closed with a rubber stopper carrying a calcium chloride tube and let stand for twelve hours at room temperature. Ice was added and the benzene layer was separated and evaporated to dryness. The residue on crystallization from alcohol gave a product melting at 131°; the alcoholic solution gives a violet color with ferric chloride.

Anal. Calcd. for $C_{20}H_{30}O_2$: C, 79.41; H, 9.99. Found: C, 79.64; H, 10.07.

Hydrocarbon $C_{19}H_{32}$.—A 50-mg. portion of the ketone $C_{19}H_{30}O$ was heated in alcoholic solution with semicarbazide hydrochloride and sodium methylate and the crude semicarbazone obtained by precipitation with water was heated to 160° during eight hours in a sealed tube with sodium methylate. The reaction product was extracted with ether and sublimed in high vacuum; the purified material melted at 55°.

Anal. Calcd. for $C_{19}H_{32}$: C, 87.61; H, 12.39. Found: C, 87.52; H, 12.45.

Action of Hydrochloric Acid on Propeimin.—A solution of 100 mg. of propeimin in alcohol was saturated with hydrogen chloride gas and then evaporated to dryness on the water-bath. The residue on crystallization from acetone melted at 167°.

Anal. Calcd. for $C_{19}H_{30}O$: C, 83.15; H, 11.02. Calcd. for $C_{20}H_{32}O$: C, 83.27; H, 11.18. Calcd. for $C_{21}H_{34}O$: C, 83.38; H, 11.33. Found: C, 83.25; H, 11.16.

Summary

In a study of the constituents of the Chinese drug *Fritillaria roylei* (Pei-Mu), further analyses of the known alkaloids peimine and peiminine indicate that the formulas should be revised to $C_{27}H_{45}O_3N$ and $C_{25}H_{41}O_3N$, respectively. In addition to these alkaloids, the author has isolated from the drug a new neutral principle of the formula $C_{26}H_{44}O_3$ or $C_{27}H_{46}O_3$ for which the name propeimin is suggested. Propeimin yields a C_{19}-ketone on oxidation and is cleaved by hydrochloric acid to a C_{19}-C_{21} product. It is suggested that the alkaloids of Pei-Mu may be related to known steroid alkaloids and propeimin to the steroid sapogenins.

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