at room temperature overnight, then at −10° for 24 hours; yield, 53.1 Gm. (32.6%), m.p. 116–119°. Purity of this material was sufficient for the succeeding reaction to form fluorodopan. Product IV may be purified by suspending it in boiling ethyl acetate (10 Gm., 250 ml.), followed by the dropwise addition of methanol until complete dissolution occurs. The melting point of pure IV was 123–123.5°.

Anal.—Calcd. for $C_9H_{14}FN_3O_3$: C, 46.7; H, 6.1; F, 8.2; N, 18.2. Found: C, 46.9; H, 6.0; F, 8.1; N, 18.0.

5 - [(2 - Fluoroethyl)(2 - chloroethyl)amino]-5-methyluracil (V).—To a stirred solution of 800 ml. of 1,2-dimethoxyethane, 21 ml. of absolute ethanol, and 0.5 ml. of water, was added 28.6 ml. (0.39 mole) of thionyl chloride. After 5 minutes, the solution was cooled to 0°, and 80 Gm. (0.35 mole) of IV was added, then 89.6 ml. (1.23 moles) of thionyl chloride (5). The suspension was stirred at 0–5° for 6 hours, then permitted to warm to room temperature. The suspension was stirred an additional 18 hours, then filtered. The product filter cake was washed by resuspending in benzene (5 × 500 ml.) and recovered by filtration; yield, 89 Gm. (83.2%), m.p. 130–140°. The product hydrochloride was recrystallized from ethanol by the addition of ethyl ether, m.p. 159–160°. Since purity of the recrystallized product was poor, the free base was liberated by neutralizing a water suspension of the salt with saturated aqueous sodium bicarbonate; yield from 89 Gm., 67 Gm. (86.2%). The free base was purified by crystallization from ethyl acetate, m.p. 166–167°.

Anal.—Calcd. for $C_9H_{13}ClFN_3O_2$: C, 43.3; H, 5.3; Cl, 14.2; F, 7.6; N, 16.8. Found: C, 43.3; H, 5.3; Cl, 14.3; F, 7.7; N, 17.1.

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Phytochemical Investigation of *Piper chaba*

By S. S. MISHRA and J. P. TEWARI

A phytochemical investigation was conducted on *Piper chaba* Hunter. β-Sitosterol, piperine, and piplartine have been isolated in pure form from the stems of this plant and identified.

PIPER CHABA Hunter (*Piperaceae*), known as chaba, is a glabrous, fleshy plant. It is cultivated in various parts of India and Malaya. The assumed medicinal effects of this plant are briefly described by Kirtikar and Basu (1), Chopra (2), and Nadkarni (3). The phytochemical study was initiated primarily because preliminary screening tests indicated that *P. chaba* possessed hypotensive and smooth muscle relaxant activity as shown in acute experiments (4). This observation and a survey of the available literature, which revealed no apparent report on the exact chemistry of this plant, prompted this investigation.

β-Sitosterol, piperine, and a new alkaloid—piplartine (m.p. 124°)—have been isolated from the petroleum ether extract of the stems of *P. chaba* and identified. This finding in the *Piper* family is not unusual (5). Piplartine has also been isolated from the stems of *P. longum* Linn., by Atal and Banga (6); its structure has recently been elucidated (7) as piperidine amide of 3,4,5-trimethoxycinnamic acid. Piperlongumine, having the melting point of piplartine and identical to it in many respects, has also been isolated from the roots of the same species by Chatterjee and Dutta (8). They proposed its structure as piperidone amide of 3,4,5-trimethoxycinnamic acid and confirmed it by synthesis, disproving the structure claimed by Atal and Banga.

EXPERIMENTAL

Plant Material.—The chemical studies were conducted on an authentic sample of stems of *P. chaba* Hunter, free of adulteration and foreign materials, obtained from M/S Jai Bharat Traders, Lucknow, India. The stems were comminuted to a No. 40 mesh powder.

Extraction Studies.—Suitable accurately weighed samples of powdered material were extracted successively with a series of solvents in a Soxhlet continuous extraction apparatus by the Rosenthaler method (9). The percentage of extractives were: petroleum ether (b.p. range 40–60°), 0.8%; solvent ether, 0.5%; chloroform, 0.5%; ethanol, 3.2%; 70% ethanol, 3.8%. Ash value, determined by the B.P. (1963), was 4.5% of the dry weight of the crude drug.

Two kilograms of the powdered drug was extracted with light petroleum, b.p. 40–60°, in a Soxhlet apparatus until an aliquot of the colorless percolate left no residue when evaporated to dryness. On keeping the extract overnight, a white deposit was obtained and separated by filtration. On further concentration and standing overnight, a further greenish-yellow deposit, which was again separated by filtration, was obtained. On removal of the solvent, the filtrate yielded a fatty residue.

Isolation and Purification of Piplartine.—The white deposit obtained above was crystallized from methanol. Silky white needles of piplartine, m.p. 124°, were obtained on three crystallizations from methanol in poor yield. It was insoluble in water and in dilute acids and alkalies but dissolved in concentrated acid and alkalies. With concentrated sulfuric acid, it turned yellow. It was soluble in chloroform and sparingly soluble in ethanol, methanol, and benzene. It did not reduce Fehling's solution even after hydrolysis. Piplartine decolorized an aqueous permanganate and bromine in glacial acetic acid. It gave positive test for nitrogen and for alkaloid with various alkaloidal reagents.

Anal.—Calcd. for $C_{17}H_{19}NO_6$: C, 64.35; H, 5.99; N, 4.41. Found: C, 64.6; H, 5.82; N, 4.40.

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The mixed melting point remained unaltered with an authentic sample of piplartine.

Isolation of Piperine.—The greenish-yellow deposit was dissolved in methanol and filtered hot. On cooling, more piplartine was obtained as fine needles, which were separated. The mother liquor was chromatographed over Brockmann alumina using benzene and benzene–chloroform mixture (1:3). Elution with benzene gave a steroid, while elution with benzene and chloroform mixture yielded piperine. After several crystallizations from benzene, light yellow crystals of piperine, m.p. 128–129°, were obtained. The test with concentrated sulfuric acid and gallic acid for methylene dioxy group was positive. A mixed melting point with an authentic specimen of piperine was not depressed.

Isolation of β-Sitosterol from Fatty Residue.—The fat was hydrolyzed with 0.5 *N* alcoholic potassium hydroxide, and the mixture of phytosterols was extracted with ether (10). The sterol mixture obtained after removal of solvent was chromatographed over alumina. The column was eluted with benzene and benzene–chloroform mixture (1:3). The benzene eluent was too small for further study. The residue obtained from benzene–chloroform mixture gave a positive Liebermann-Burchard test. On several crystallizations from methanol, the residue gave colorless needles, m.p. 136°, $(\alpha)_D^{25}$ −36.5° ($CHCl_3$). The sterol was freely soluble in benzene, chloroform, and petroleum ether and sparingly soluble in cold methanol and ethanol.

Anal.—Calcd. for $C_{29}H_{50}O$: C, 84.05: H, 12.09. Found: C, 83.84; H, 12.13.

The sterol acetate, benzoate, and digitonide were prepared in the usual manner; m.p. 127, 144, and 221° dec., respectively. β-Sitosterol and its acetyl and benzoyl derivatives did not show a change in melting point when admixed with the respective authentic specimen.

Thus, from a comparison of the data with those for known sitosterol, this sterol was identified as β-sitosterol. The characteristics of the sterol are in conformity with the earlier observation of the authors (11) on the sterol from *Ipomoea degitata* Linn. (*Convolvulaceae*).

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Thimerosal as a Preservative in Biological Preparations. III. Factors Affecting the Concentration of Thimerosal in Aqueous Solutions and in Vaccines Stored in Rubber-Capped Bottles

By J. BIRNER and J. ROS. GARNET

Inactivation of the antiseptic properties of thimerosal contained in vaccines stored in bottles which have been sealed with rubber closures is governed by the type of rubber used for sealing the containers, the temperature and duration of storage, and the ratio of volume of liquid to surface area of the rubber to which the thimerosal is exposed.

THE POLAROGRAPHIC method of estimating thimerosal[1] in dilute solutions has been applied to its determination in samples of pertussis vaccine and triple antigen[2] during storage of the preparations in contact with rubber closures of differing compositions for varied periods of time and temperature. The purpose of this investigation was to assess the suitability of thimerosal as a preservative in these vaccines and to provide a basis for their storage under optimum conditions.

There is no question that the preservative effect of thimerosal in the low concentration employed in biological preparations is appreciably diminished when those preparations are stored in containers sealed with rubber closures (1).

Considerations of safety in the use of vaccines dispensed in multidose containers which have been sealed with rubber caps make it important to know at what stage in the shelf life of the preparation the concentration of the preservative may be expected to fall below what can be regarded as an effective level.

As shown in a previous paper (2) the determination of thimerosal with the polarograph can be carried out rapidly and with no less accuracy than is possible with the customarily used biological method.

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[1] Thimerosal, sodium ethylmercurithiosalicylate, is official in the "British Pharmacopoeia" as Thiomersal. It is trademarked as Merthiolate.

[2] Triple antigen is an alternate name for diphtheria, tetanus, and pertussis vaccine B.P.