Synthesis of 5-[(2-Fluoroethyl)(2-chloroethyl)amino]-6-methyluracil (Fluorodopan)

By HENRYK DUBICKI, FLORIAN ZIELINSKI, and FRED W. STARKS

Fluorodopan has been synthesized and its antitumor activity compared to 5-[2-(bis-chloroethyl)amino]-6-methyluracil (dopan).

LARIONOV (1) describes the excellent activity of fluorodopan as a chemotherapeutic agent for cancer. However, the author indicates no preparative method. This paper reports the synthesis of this compound as performed in our laboratory.

Fluorodopan has shown activity[1] against the Walker 256 (subcutaneous) tumor system and against the Walker 256 (intramuscular) tumor system. In the former system, the therapeutic index was equal to 4, compared to 13 for dopan (2); in the latter system, fluorodopan has shown complete tumor inhibition in three cases. However, the doses required to produce this effect also reflected considerable toxicity to the host in both weight loss and death. Minimal activity was displayed by fluorodopan against the Dunning leukemia system. This was the same response for dopan. No reproducible activity was seen when fluorodopan was tested against the sarcoma 180, lymphoid leukemia 1210, and 9KB cell culture cytotoxicity test systems.

EXPERIMENTAL

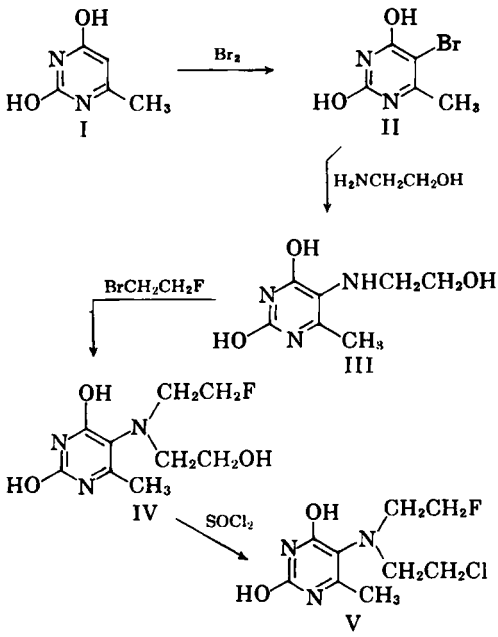
5-Bromo-6-methyluracil (II).—To 150 Gm. (1.19 moles) of 6-methyluracil (I) contained in a 2-L. round-bottomed flask was added 240 Gm. (1.50 moles) of liquid bromine. The mass was stirred manually at room temperature to cause intimate contact of bromine with the solid uracil. When the product had changed to a homogeneous red color, the mass was powdered. The flask was evacuated on a flash evaporator at 90° to remove excess bromine and hydrogen bromide. A heating period of 2–3 hours was required to cause the reaction material to become a constant yellow-white. The product was washed in 4 L. of boiling water for 20 minutes, cooled to 20°, and recovered by filtration. The yield, after recrystallizing from water, was 209.5 Gm. (86%), m.p. 230–237° [lit. (3) m.p. 230°].

Anal.—Calcd. for $C_5H_5BrN_2O_2$: C, 29.3; H, 2.5; Br, 39.0; N, 13.7. Found: C, 29.4; H, 2.5; Br, 39.1; N, 13.6.

5-Hydroxyethylamino-6-methyluracil (III).[2]—A 1-L. steel autoclave was charged with 240 Gm. (1.17 moles) of II, 276 Gm. (4.53 moles) of 2-aminoethanol, and 120 ml. of water. The mixture was agitated manually at room temperature until a homogeneous paste formed. The autoclave was sealed and heated at 160° for 5 hours, then cooled to room temperature in a water bath. A clear brown oil was obtained, which was dissolved in 2 L. of methanol and cooled to −20°, causing the crystalline product to precipitate. The product was decolorized with Norit and recrystallized from methanol to yield 85.7 Gm. (39.6%), m.p. 183–184°.

Anal.—Calcd. for $C_7H_{11}N_3O_3$: C, 45.4; H, 6.0; N, 22.7. Found: C, 45.3; H, 6.0; N, 22.7.

5 - [(2 - Fluoroethyl)(2 - hydroxyethyl)amino]-6-methyluracil (IV).—A 2-L. round-bottomed flask, fitted with a water-cooled condenser, was charged with 350 ml. of dimethylformamide and 131 Gm. (0.708 mole) of III. The mixture was stirred magnetically and heated to 90°. Bromoethylfluoride (90 Gm., 0.708 mole) and cyclohexene oxide (64.4 Gm., 0.708 mole) were added to the reaction mixture in equal portions during eight 3-hour intervals. After the reaction mixture had been heated a total of 27 hours, the dimethylformamide was removed *in vacuo* to leave a dark-red oil. The oil was dissolved in a minimum amount of methanol (about 100 ml.) and added to 2.0 L. of ethyl acetate, causing the precipitation of a red-black oil fraction which was discarded. The supernatant ethylacetate solution was evaporated *in vacuo* to a light-colored oil which was triturated with boiling ethyl acetate (10 × 200 ml.). The combined ethyl acetate solutions were concentrated *in vacuo* to about 500 ml., heated to boiling, and decanted from a yellow oil which had been deposited. The hot ethyl acetate was seeded with authentic product[3] IV, cooled at a rate of 20°/hour, stored



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[1] These data were obtained from the Screening Section, Drug Evaluation Branch, Cancer Chemotherapy National Service Center, National Cancer Institute.

[2] The method is similar to that employed for the production of 5-(2-hydroxyethylamino) uracil (4).

[3] Seed crystals were obtained by a chromatographic separation of the reaction residue on activated alumina. When eluted with ethyl acetate, the desired fraction produced a white fluorescence (U.V.) on filter paper. When the color turned blue, collection of the fraction was stopped. Concentration of the white fluorescing fraction *in vacuo* produced a crystalline product (IV).

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.

REFERENCES

(1) Larionov, L. F., and Sof'ina, Z. P., *Dokl. Akad. Nauk SSSR*, **114**, 1070(1957).
(2) Larionov, L. F., *Nestnik Akad. Med. Nauk SSSR*, **14** 25(1959).
(3) Behrend, R., *Ann.*, **229**, 18(1885).
(4) Baker, B. R., *J. Am. Chem. Soc.*, **82**, 4585(1960).
(5) Lyttle, D. A., and Petering, H. G., *ibid.*, **80**, 6549 (1958).

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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