22°), $[\Phi]_{400}$ +1900°, $[\Phi]_{317}$ +4350, $[\Phi]_{279}$ −890°, $[\Phi]_{253}$ +1580°, $[\Phi]_{242}$ +890°, $[\Phi]_{231}$ +5450°. *Anal.* ($C_{10}H_{13}NO_4$) C, H, N.

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Fluorinated Pyrimidines. XXXVI. Synthesis of Some 2,4-Substituted 5-Trifluoromethylpyrimidines[1]

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In this laboratory we have been concerned for some time with 5-trifluoromethyl-2′-deoxyuridine,[4] which has powerful tumor inhibitory[5] and antiviral activities.[6,7] 5-Methyl-2′-deoxycytidine has been found in mammalian and polyoma viral DNA.[8] Even though it is known that the methylation of 2′-deoxycytidine occurs after its incorporation into DNA, we were interested in preparing the corresponding trifluoromethyl derivative and testing it for biological activity. Because of the alkaline instability of the trifluoromethyl group in 5-trifluoromethyluracil[4] and its nucleosides, conventional nucleoside syntheses that require alkaline deblocking were considered unsuitable. Consequently, we attempted to prepare 5-trifluoromethyl-2′-deoxycytidine enzymatically with the *trans*-N-deoxyribosylase[9] from *Lactobacillus helveticus* (ATCC 8018) by an exchange between 5-trifluoromethylcytosine, prepared from 2,4-dichloro-5-trifluoromethylpyrimidine,[10] and a suitable deoxyribonucleoside donor. This enzymatic route has been used successfully in the syntheses of the analogs, 5-trifluoromethyl-2′-deoxyuridine[4] and 5-nitro-2′-deoxyuridine.[11] Although the synthesis of 5-trifluoromethyl-2′-deoxyuridine could be accomplished with our enzyme preparation, we were not able to detect by tlc the presence of 5-trifluoromethyl-2′-deoxycytidine in any attempted enzymatic reaction between 5-trifluoromethylcytosine and either thymidine, 2′-deoxycytidine, 2′-deoxyuridine, 2′-deoxyguanosine, or 2′-deoxyadenosine. Therefore, although cytosine was readily converted to 2′-deoxycytidine with this enzyme, 5-trifluoromethylcytosine apparently had no affinity for the enzyme.

For other reasons, 4-benzylamino-2-hydroxy-5-trifluoromethylpyrimidine (VIII) was synthesized from 2,4-dichloro-5-trifluoromethylpyrimidine (I). Two isomers, 4-benzylamino-2-chloro-5-trifluoromethylpyrimidine (IV) and 2-benzylamino-4-chloro-5-trifluoromethyl pyrimidine (V), were obtained which could only be separated by tlc. On treatment with NaOMe the mixture of IV and V gave the corresponding isomeric methoxy compounds, VI and VII, which could be separated by mechanical retrieval of two distinct crystal forms. The structures of the isomers were determined as follows. On hydrolysis of IV and V, as well as VI and VII, with 1 *N* HCl, two of the compounds were more reactive and should have had the Cl and OCH_3 groups in the 2 position. Furthermore, 2-benzylamino-4-chloro-5-trifluoromethylpyrimidine had a greater bathochromic shift in its spectrum relative to the 2,4-dichlorocompound than did its isomer, which is in agreement with analogous studies by Boarland and McOmie.[12]

Biological Activity.—Compounds II and III did not inhibit the growth of L5178Y cells in culture[13] at 10^{-4} *M*. However, compounds VI and VII inhibited these cells approximately 50% at 10^{-4} *M*.

Experimental Section

All melting points are corrected. All analyses are by Galbraith Laboratories, Knoxville, Tenn. All uv spectra were determined on a Cary Model 15 spectrophotometer.

2,4-Dichloro-5-trifluoromethylpyrimidine (I) was synthesized as described by Shen, *et al.*[10]

4-Amino-2-chloro-5-trifluoromethylpyrimidine (II).—To 1.0 g of I in a precooled steel bomb (Parr) was added 20 ml of liquid NH_3. The bomb was closed and gradually allowed to come to room temperature and was then recooled. After evaporation of the NH_3, the residue was extracted with 150 ml of Et_2O and filtered, and the solvent was evaporated to dryness *in vacuo*. The residue was crystallized from EtOH to give 900 mg (98%) of II, mp 146°, λ_{max}^{MeOH} 239 and 282 mμ (ε 21,000 and 2500). *Anal.* ($C_5H_3F_3ClN_3$) C, H, N.

4-Amino-2-hydroxy-5-trifluoromethylpyrimidine (III).—Compound II (900 mg) was refluxed in 100 ml of 0.1 *N* HCl until it dissolved. The solution was then evaporated *in vacuo*, and the residue was washed with EtOH and Et_2O. It was then dissolved in 100 ml of 80% EtOH and 4 g of Amberlite IR-48 (OH^-) was added. The mixture was stirred for 15 min until the pH reached 6.0. The resin was filtered and washed with EtOH, and the combined filtrates were evaporated to dryness *in vacuo*. The residue was dissolved in EtOH and reprecipitated with Et_2O to give 500 mg (61%) of a colorless solid, which was recrystallized from EtOH; mp 250–255° dec, λ_{max}^{MeOH} 286 mμ (ε 7700). *Anal.* ($C_5H_4F_3N_3O$) C, H, F, N.

Isomeric Mixture of 4-Benzylamino-2-chloro-5-trifluoromethylpyrimidine (IV) and 2-Benzylamino-4-chloro-5-trifluoromethylpyrimidine (V).—To a stirred solution of I (810 mg) in 3 ml of EtOH was added 803 mg of benzylamine in 3 ml of EtOH. The mixture was warmed; it decolorized and was kept at 40° for 15 min when a crystalline precipitate formed. The mixture was evaporated to dryness, and the residue was dissolved in 50 ml of Et_2O. The ether phase was washed with three 50-ml portions of H_2O, 50 ml of 0.1 *N* HCl, then 50 ml of H_2O, and was dried (Na_2SO_4). The Et_2O was evaporated *in vacuo* to give an oil, which solidified on standing. This material was crystallized from petroleum ether (bp 50–70°) to give 995 mg (79%) of a mixture of the two isomers IV and V. After recrystallization, an analytical sample of the mixtures was obtained, mp 101–104°. *Anal.* ($C_{12}H_9F_3ClN_3$) C, H, F.

A few milligrams of the mixture of isomers was separated by tlc (Eastman 6060) in PhH to give IV, mp 106°, and V, mp 110°. The structures of the isomers were assigned as described above.

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(4) C. Heidelberger, D. G. Parsons, and D. C. Remy, *J. Med. Chem.*, **7**, 1 (1964).

(5) C. Heidelberger and S. W. Anderson, *Cancer Res.*, **24**, 1979 (1964).

(6) H. E. Kaufman and C. Heidelberger, *Science*, **145**, 585 (1964).

(7) M. Umeda and C. Heidelberger, *Proc. Soc. Exp. Biol. Med.*, **130**, 24 (1969).

(8) E. Winocour, A. M. Kaye, and V. Stollar, *Virology*, **27**, 156 (1965).

(9) A. H. Roush and R. F. Betz, *J. Biol. Chem.*, **233**, 261 (1958).

(10) T. Y. Shen, H. M. Lewis, and W. V. Ruyle, *J. Org. Chem.*, **30**, 835 (1965).

(11) D. Kluepfel, Y. K. S. Murthy, and G. Settori, *Farmaco. Ed. Sci.*, **20**, 757 (1965).

(12) M. P. V. Boarland and J. F. W. McOmie, *J. Chem. Soc.*, 3716, 3722 (1952).

(13) M. Umeda and C. Heidelberger, *Cancer Res.*, **28**, 2529 (1968).

4-Benzylamino-2-methoxy-5-trifluoromethylpyrimidine (VI) and 2-Benzylamino-4-methoxy-5-trifluoromethylpyrimidine (VII).—The mixture of IV and V (600 mg) and 125 mg of $NaOCH_3$ were dissolved in 10 ml of MeOH and heated in a sealed tube at 100° for 20 hr. The solution was decanted from the salt and evaporated to dryness. The residue was dissolved in PhH and concentrated *in vacuo* to 5 ml, and 10 ml of petroleum ether was added. After cooling in a refrigerator two crystal forms came out, which were separated manually. After recrystallization from 1:2 PhH–petroleum ether, 213 mg of colorless needles of VII (36%) was obtained, mp 120.5°, λ_{max}^{MeOH} 243 and 279 mμ (ε 76,000 and 13,000). *Anal.* ($C_{13}H_{12}F_3N_3O$) C, H, F.

Similarly, 162 mg (27%) of colorless cubes of VI, mp 85°, was obtained: λ_{max}^{MeOH} 239 and 273 mμ (ε 29,000 and 11,000). *Anal.* ($C_{13}H_{12}F_3N_3O$) C, H, F. The structural assignment of the isomers was described above.

4-Benzylamino-2-hydroxy-5-trifluoromethylpyrimidine Hydrochloride (VIII).—Compound VI (150 mg) was refluxed for 2 hr with 4 ml of concentrated HCl. The acid was removed on the rotary evaporator *in vacuo*, followed by successive additions and evaporations of H_2O, EtOH, and 1:1 Et_2O–petroleum ether. The residue was crystallized from acetone to give 77 mg (48%) of VIII, mp 175–187° dec, λ_{max}^{MeOH} 250 mμ (ε 13,000). *Anal.* ($C_{12}H_{11}ClF_3N_3O$) C, H, F.

Preparation of Enzyme Solution.—*Lactobacillus helveticus* (ATCC 8018) was grown for 24 hr in 7 l. of medium containing 15 g of Bacto-Trypton (Difco), 5 g of yeast extract (Difco), 10 g of glucose, 2 g of KH_2PO_4, 1 ml of Tween 80, and 100 ml of fresh tomato juice per l.[11] Upon centrifugation, 18 g of cells was obtained. The cells were washed twice by centrifugation with 150 ml of 0.05 *M* potassium phosphate buffer pH 6.5 and then passed twice through a French press in 80 ml of the same buffer. The homogenate was centrifuged at 10,000 *g* for 10 min, the supernatant fraction was dialyzed against the same buffer and stored at 5°.

Enzymatic Syntheses of Nucleosides.—The donor nucleoside (1.0 mg of thymidine or 2′-deoxyadenosine) and 1.0 mg of the acceptor base (5-trifluoromethyluracil or III) were dissolved in 0.5 ml of 0.05 *M* potassium phosphate buffer, pH 5.8, added to 0.5 ml of the enzyme solution, and incubated for 3 hr at 37°. Four volumes of EtOH were added to precipitate the protein, the supernatant fraction was evaporated to 0.1 ml, and an aliquot was spotted on a thin layer sheet. After development, the tlc was inspected under uv light to locate the bases or nucleosides and sprayed with a cysteine–H_2SO_4 solution for detection of deoxyribose and deoxyribonucleosides.

Chromatography.—Eastman Chromogram sheets 6060, silica gel with fluorescent indicator, were used throughout. PhH–MeOH (3:1 v/v) was usually used; PhH was the solvent for the separation of IV from V and VI from VII. The enzymatic syntheses were followed in $CHCl_3$–MeOH (9:1 and 3:1).

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Synthesis and Activity of a New Class of Heterocyclic Compounds against *Entamoeba histolytica*. 1,2,3,3a-Tetrahydro-1-alkylcyclopenta[*de*]quinolines

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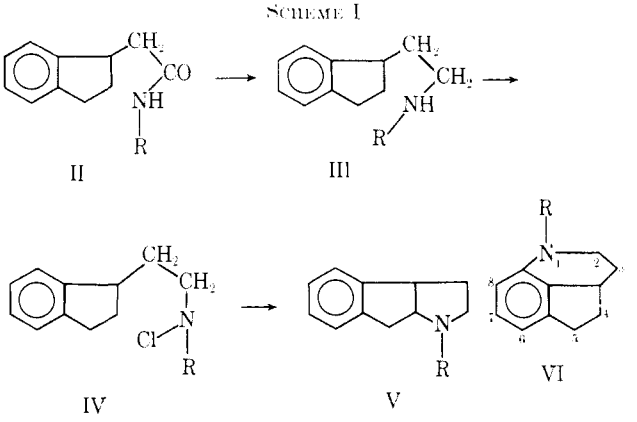
In continuation of our investigation on the synthesis of various substituted 1,2,3,3a,8,8a-hexahydroindeno[1,2-*c*]pyrroles[1-3] (I) as potent amebicidal agents, synthesis of a series of isomeric compounds like 1,2,3,3a,8,8a-hexahydro-1-alkyl[2,1-*b*]indenopyrroles (V) was undertaken by the route shown in Scheme I which ultimately led to compounds VI (Table III).

SCHEME I



Interaction of 1-indanacetyl chloride[4] and the appropriate alkylamine furnishes the amide II which was reduced to the 1-alkylaminoethylindan III with LAH in dry Et_2O. Hofmann–Löffler reaction on this secondary amine according to a procedure by Coleman, *et al.*,[5] yield a tertiary amine which analyzed for the expected amine (V, R = Me), but the nmr spectrum[6] of the amine shows the ratio of aromatic protons to non-aromatic protons as 1:4. In compound V the above ratio is 1:2.75, whereas the same ratio in compound VI (R = Me) is 1:4. On this basis the structure of the tertiary amine has been designated VI.

The *in vitro* amebicidal activity of the hydrochlorides of VI is very poor. None of these compounds is active at a concentration of 100 μg/ml, while emetine hydrochloride is active at a concentration of 1 part in 256,000.[7]

Experimental Section[8]

1-Alkylacetamidoindans (II) were prepared by the interaction of the appropriate alkylamine (1.5 moles) and 1-indanacetyl chloride[4] (1 mole) under stirring in the presence of 2.5 *N* NaOH at 10–15° for 1.5 hr in almost quantitative yield. They were either crystallized from PhH–petroleum ether (bp 60–80°) or distilled. Physical properties are reported in Table I.

1-Alkylaminoethylindans (III).—The appropriate amide (1 mole) was reduced with LAH (1.2 moles) in dry Et_2O for 12–16 hr in 70–80% yield. Their characteristics are shown in Table II.

1,2,3,3a-Tetrahydro-1-alkylcyclopenta[*de*]quinolines (VI).—In an ice-cold mixture of III (R = Me; 5 g, 28.6 mmoles), petroleum ether (bp 60–80°, 21 ml), and 3 *N* NaOH (21 ml), Cl_2 was passed with stirring till the white fumes of amine hydrochloride disappeared. The greenish yellow petroleum ether layer was separated out, washed successively (cold 3 *N* NaOH, 3 ml; ice water, 3 ml; cold 2 *N* H_2SO_4, 3 ml), and stirred in an ice bath with a mixture of 98% H_2SO_4 (12 ml) and H_2O (5 ml) for 10 min. The acid layer was separated out and the petroleum ether layer was extracted twice (cold 98% H_2SO_4, 4 ml). The (H_2SO_4) extracts were further admixed with 98% H_2SO_4 (2.5 ml) and H_2O (1 ml) and

(1) K. L. Pathak and B. Pathak, *J. Indian Chem. Soc.*, **38**, 253 (1961).
(2) A. U. Dey and B. Pathak, *Indian J. Chem.*, **2**, 371 (1964).
(3) A. U. De and B. Pathak, *ibid.*, **3**, 93 (1965).
(4) S. C. Lahiri and N. C. De, *J. Med. Chem.*, **11**, 900 (1968).
(5) G. H. Coleman, G. Nichols, and T. F. Martens, *Org. Syn.*, **25**, 14 (1945).
(6) Nmr analyses were carried out on a Varian HA 100 nmr spectrometer and were calibrated against TMS.
(7) Amebicidal screening of the compounds were carried out by the Central Drug Research Institute, Lucknow, India.
(8) Melting points were determined by the capillary tube method in a Gallenkamp apparatus and are corrected. Boiling points are uncorrected. All compounds were analyzed for C, H, N. Analytical data were within ±0.4% of theoretical values. Uv absorption spectra were measured on a Beckmann spectrophotometer Model D.U. in absolute ethanol.