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.[10] 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).

Acknowledgment.—We are indebted to Miss Sheryl Kessler for carrying out the spectrophotometric analyses and to Dr. Yoshisada Fujiwara for the cell culture experiments.

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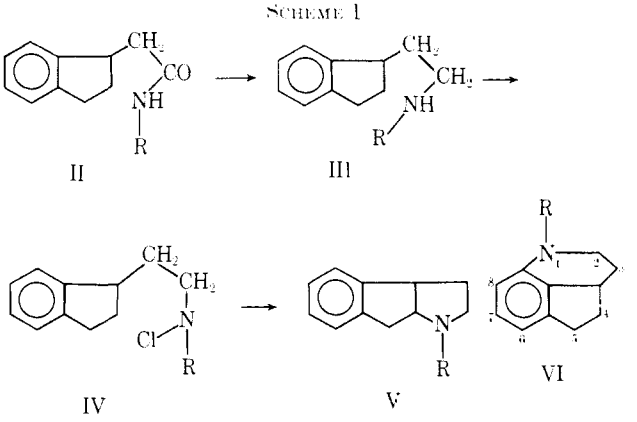
In continuation of our investigation on the synthesis of various substituted 1,2,3,3a,8,8a-hexahydroindeno[1,2-*c*]pyrroles[1-3] (I) as potent amebicidal agents, synthesis of a series of isomeric compounds like 1,2,3,3a,8,8a-hexahydro-1-alkyl[2,1-*b*]indenopyrroles (V) was undertaken by the route shown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

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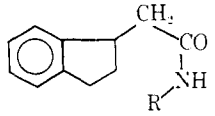
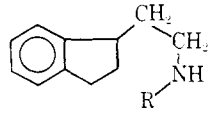
(6) Nmr analyses were carried out on a Varian HA 100 nmr spectrometer and were calibrated against TMS.
(7) Amebicidal screening of the compounds were carried out by the Central Drug Research Institute, Lucknow, India.
(8) Melting points were determined by the capillary tube method in a Gallenkamp apparatus 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.

TABLE I
1-ALKYLACETAMIDOINDANS (II)



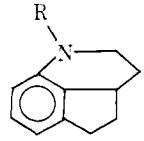
R	Mp, °C	Bp, °C (mm)	Formula
Me	72–73		$C_{12}H_{15}NO$
Et	69–71		$C_{13}H_{17}NO$
n-Pr		185–187 (1)	$C_{14}H_{19}NO$
n-Bu		173–175 (0.8)	$C_{15}H_{21}NO$

TABLE II
1-ALKYLAMINOETHYLINDANS (III)



R	Bp, °C (mm)	Formula	λ_{max}, mμ	ε × 10^{-2}
Me	90–92 (0.5)	$C_{12}H_{17}N$	266, 273	11.43, 12.43
Et	104–105 (0.6)	$C_{13}H_{19}N$	266, 273	11.89, 13.03
n-Pr	125–127 (1)	$C_{14}H_{21}N$	266, 273	11.42, 9.88
n-Bu	135–137 (0.8)	$C_{15}H_{23}N$	266, 273	11.63, 10.18

TABLE III
1,2,3,3a-TETRAHYDRO-1-ALKYLCYCLOPENTA[*de*]QUINOLINES (VI)



R	Bp, °C (mm)	Formula	λ_{max}, mμ	ε × 10^{-2}
Me	83–85 (0.5)	$C_{12}H_{15}N$	265	67.59
Et	114–115 (0.6)	$C_{13}H_{17}N$	268	64.15
n-Pr	120–122 (0.8)	$C_{14}H_{19}N$	267	38.04
n-Bu	107–109 (1)	$C_{15}H_{21}N$	262	31.72

the mixture was heated at 70–80° with stirring for 0.5 hr in the presence of light. It was then cooled and poured onto ice, basified with NaOH under cooling, extracted (PhH), and tosylated with TsCl (6 g, 31.6 mmoles) in PhH solution under stirring at 5–8° in the beginning and later at 40° with simultaneous addition of 3 *N* NaOH (25 ml) to keep the mass alkaline. The PhH layer was separated out and the tertiary amine was repeatedly extracted (6 *N* HCl). The combined acid extracts were basified with NaOH under cooling, extracted (Et_2O), and dried (Na_2SO_4), and the base was distilled. The yield varied from 30–40%. The physical characteristics of VI are reported in Table III.

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Antimalarials. 4-Substituted 1H-Pyrazolo[3,4-*b*]quinolines

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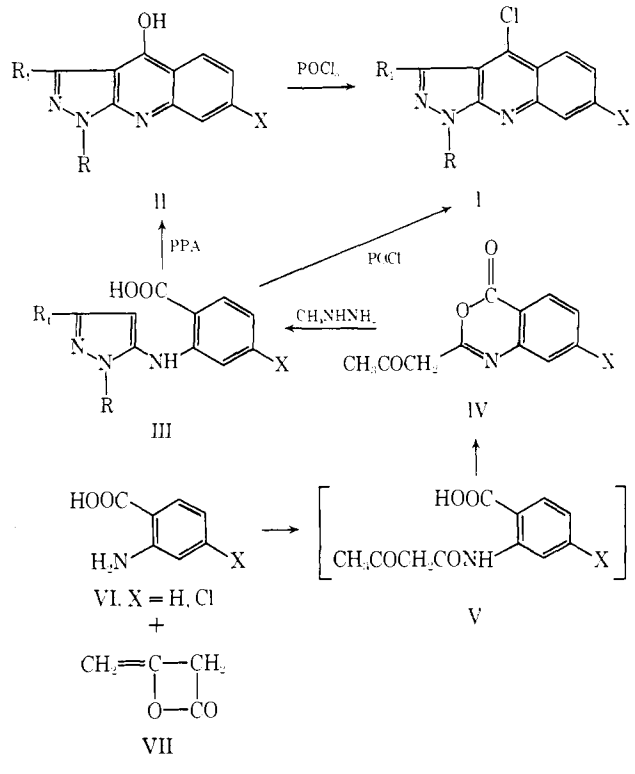
Pyrazole derivatives are known to possess various kinds of biological activity. For example, the pyrazolo[3,4-*b*]pyrimidine derivative, an isostere of caffeine, is indistinguishable from caffeine in its diuretic properties and is also a strong CNS stimulant.[1] 5-Aminopyrazolo[3,4-*b*]pyridines are vasodilators or cardiotonics.[2] 1-Substituted 3-dimethylaminoalkoxy-1H-indazoles show sedative, muscle relaxant, and antiinflammatory properties.[3] Several pyrazole derivatives, where the pyrazole ring is not fused with another ring, such as substituted aminopyrazoles, possess antiinflammatory, analgetic, antipyretic, adrenolytic, narcosis-potentiating, and antirheumatic activity.[4] Several derivatives of 1-phenyl-3-methyl-4-(substituted amino)-1H-pyrazolo[3,4-*b*]quinolines (anilino and substituted anilino)[5] and 1,3-dimethyl-1H-pyrazole[3,4-*b*]quinoline[6,7] have been prepared but not tested.

We were interested in combining the features of the pyrazole ring, a substituted quinoline, and an "antimalarial" side chain in one molecule for antimalarial testing. The key intermediate required was a 4-chloro-1H-pyrazolo[3,4-*b*]quinoline (I), in which the active Cl could be replaced with suitable amines expected to impart antimalarial activity to the final products. The method for preparing it is outlined in Scheme I.

SCHEME I



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