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Keyphrases

Antiradiation compounds
Nitriles, aminonitriles—sulfur derivatives
Paper chromatography—identity
IR spectrophotometry—structure
UV spectrophotometry—structure

Pyrrolo [2,3-*d*]Pyrimidines

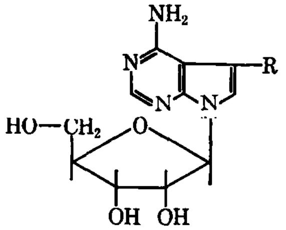
By RICHARD H. HAMMER

The synthesis of a series of 4-alkylamino-7-methyl analogs of tubercidin from 4-chloro-7-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidine is described.

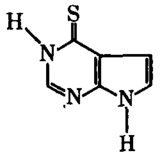
TUBERCIDIN (4-amino-7-β-D-ribofuranosyl-7*H*-pyrrolo[2,3-*d*]pyrimidine) (I) and two other closely related naturally occurring nucleosides containing the pyrrolo[2,3-*d*]pyrimidine structure, toyocamycin (II), and sangivamycin (III), show pronounced cytotoxic activity (1–3). As a result of this activity and the unique stability of the base-sugar bond to enzymatic (4) and *in vitro* acid cleavage,[1] considerable interest in the synthesis and evaluation of tubercidin analogs has developed.

Recently, Gerster *et al.* (5) synthesized a series of 4-substituted derivatives of tubercidin with the ribofuranosyl group intact on the 7 position. Montgomery and Hewson (6) have observed a decrease in cytotoxic activity, compared to tubercidin, when substituting aliphatic and cycloaliphatic groups at the 7 position with a free amino group in the 4 position. Cell-culture cytotoxicity studies on Compounds IV and V (Table III), synthesized in this laboratory (7), indicate an increase in activity when the bulky, lipophilic benzyl group is substituted on the mercapto group of IV. These observations prompted the synthesis of a series of 4-alkylamino-7-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidines (VIII*a*-VIII*f*) where the series extends from the simpler alkyls, such as ethyl, to heterocyclic rings such as pyrrolidyl and piperidyl. In order to simulate the pKa of tubercidin, the described compounds all have a methyl group substituted on the 7-nitrogen.

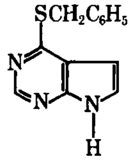
Synthesis of these compounds has been accomplished through methylation of 4-chloro-7*H*-pyrrolo-[2,3-*d*]pyrimidine (VI) with methyl iodide (8) and reaction of this 7-methyl product (VII) with the appropriate amine in a Parr bomb at elevated temperatures to give the respective 4-alkylamino-7-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidine compound (VIII*a*–VIII*f*).



I, R = H
II, R = C≡N
III, R = $\overset{O}{\overset{\|}{C}}—NH_2$



IV



V

EXPERIMENTAL

Melting points were obtained on a Thomas-Hoover capillary melting-point apparatus and are uncorrected. Ultraviolet analysis was recorded on a Beckman model DB spectrophotometer. Titrimetric pKa values were obtained on a Sargent titrator, model D at 25°. NMR spectra were obtained on a Varian A-60 instrument at a field strength of 60 Mc./sec. Microanalysis was conducted by Galbraith Laboratories, Inc., Knoxville, Tenn.

Method A—To 0.175–0.20 g. of VII (8) was

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[1] Preliminary degradation studies in this laboratory show tubercidin to be completely stable for 66 hr. in 0.1 *N* HCl at 80° compared to a half-life of 72 min. for adenosine under identical conditions.

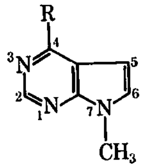
added a 1-mole excess of alkylamine and 15–25 ml. of 95% ethanol. The solution was heated with stirring in a Parr bomb at 125–130° for 6–6.5 hr. The reaction mixture was acidified to approximately pH 2 with 20% HCl, filtered, and the filtrate adjusted to pH 10 with 20% NaOH. The mixture was evaporated to dryness and the resultant oily syrup was taken up in water–chloroform and subsequently extracted with chloroform. Removal of the chloroform left a syrup which was purified using preparative thin-layer chromatography to yield crystalline, analytically pure samples (1-mm. Silica Gel GF_{254}; solvent system, ethyl acetate–methanol, 9:1). Including fluorescent zones on the starting line and solvent front, four bands were observed under short wavelength UV light. Extraction of the second band from the solvent front with 95% ethanol and evaporation of the solvent yielded the products which were characterized as the free base and/or the HCl salt. See Tables I and II. In the case of VIII*b*, after extraction from the plate and removal of the solvent, it was necessary to triturate with petroleum ether to obtain the product.

Method B—After heating the reactants in a Parr bomb at 125–130° for 6–6.5 hr. as outlined under Method A, the solution was concentrated to a viscous oily syrup and purified *via* two consecutive preparative thin-layer chromatography steps (Method A) to give the final product which was analyzed as the free base and/or the HCl salt. See Tables I and II.

RESULTS AND DISCUSSION

The five-step procedure of Davall's (9) led to the 4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine intermediate (VI) which was converted to the 7-methyl analog (VII) by methylation with methyl iodide (8). Nucleophilic displacement of the 4-chloro group of VII and other 4-chloro-7-alkyl-7*H*-pyrrolo[2,3-*d*]-pyrimidines with methanolic ammonia to give the

TABLE I—PHYSICAL PROPERTIES OF



Compd.[a]	R	Method	% Yield	M.p., °C. Base	M.p., °C. HCl	Molecular Formula	Anal. Calcd	Anal. Found	pKa[b]
VIII*a*	4-Ethylamino	A	52	156–58[c]	233–34 dec.	$C_9H_{12}N_4$	C 61.37 H 6.81 N 31.82	C 61.25 H 6.95 N 31.67	5.64
VIII*b*	4-*n*-Propylamino	A B	32 50	118–20	203–04 dec.	$C_{10}H_{14}N_4$	C 63.16 H 7.36 N 29.47	C 63.00 H 7.48 N 29.24	5.52
VIII*c*	4-*n*-Pentylamino	B	59	72–3[d]	67–9	$C_{12}H_{18}N$	C 66.04 H 8.33 N 25.68	C 65.85 H 8.08 H 25.53	5.66
VIII*d*	4-*n*-Hexylamino[e]	B	57	71–2	158–60	$C_{13}H_{20}N_4$	C 67.06 H 8.69 N 24.14	C 65.78 H 8.63 N 23.71	—
VIII*e*	4-(1-Pyrrolidyl)[e]	B	80	99–100	[f]	$C_{11}H_{14}N_4 \cdot HCl$	C 55.37 H 6.45 N 23.48	C 54.34 H 6.36 N 23.00	—
VIII*f*	4-(1-Piperidyl)	B	59[g]	—	195–96 dec.	$C_{12}H_{18}N_4 \cdot HCl$	C 57.04 H 7.59 N 22.17	C 56.81 H 7.28 N 21.95	5.28

[a] The 4-*n*-butyl compound was also prepared. M.p. (free base) 112–113°. Calcd. for $C_{11}H_{16}N_4$: C, 64.71; H, 7.87; N, 27.44. Found: C, 64.57; H, 7.90; N, 27.20. [b] Dissociation constants were determined on the HCl salts in distilled water at 25°. [c] Reported: 159° (10). [d] Reported 125–127° (10). See *Results and Discussion.* [e] Compounds VIII*d* and VIII*e* were not within acceptable limits for carbon analysis. Insufficient amounts of both samples prohibited further analysis. [f] Begins darkening around 240° and melts with decomposition at 260°. [g] Calculated as the HCl salt from starting material VII.

TABLE II—UV AND NMR ANALYSIS

Compd.	λmax. mμ (ε) pH 1.0[a]	λmax. mμ (ε) Ethanol	λmax. mμ (ε) pH 13.0	Compd.	NMR (τ, p.p.m.) Protons NHCN	NMR (τ, p.p.m.) Protons =CH	NMR (τ, p.p.m.) Protons NCH_3	NMR (τ, p.p.m.) Protons NCH_2	NMR (τ, p.p.m.) Protons CH_2 & CH_3	$J_{5,6}$ c.p.s.
VIII*a*	232 (19,370) 274 (12,360)	273 (12,150)	273 (11,970)	VIII*a*[b]	1.93	2.82 3.38	6.28	6.52	8.71 (CH_3)	3.5
VIII*b*	232 (19,020) 274 (13,120)	276 (13,310)	275 (12,930)							
VIII*c*	233 (20,140) 274 (12,940)	276 (12,730)	276 (12,940)							
VIII*d*	232 (18,580) 274 (11,840)	278 (9,750)	275 (12,770)	VIII*c*[c]	1.83	2.88 3.41	6.28	6.51	8.56 9.09 complex, overlapping	3.5
VIII*e*[d]	237 (12,600) 280 (9,700)	281 (9,450)	282 (9,390)	VIII*d*[e]	1.65	3.12 3.66	6.20	6.43	8.08 8.62 9.12 9.94	3.5
VIII*f*[d]	238 (13,640) 284 (11,620)	288 (16,170)	290 (12,880)							

[a] pH 1.0 (0.1 *N* HCl); ethanol, 95%; pH 13.0 (0.1 *N* NaOH). [b] HCl salt in water-*d* with tetramethylammoniumtetrafluoroborate as the internal standard. [c] Determined in d_6-dimethylsulfoxide. [d] HCl salt. [e] Determined in chloroform-*d* with tetramethylsilane as the internal standard.

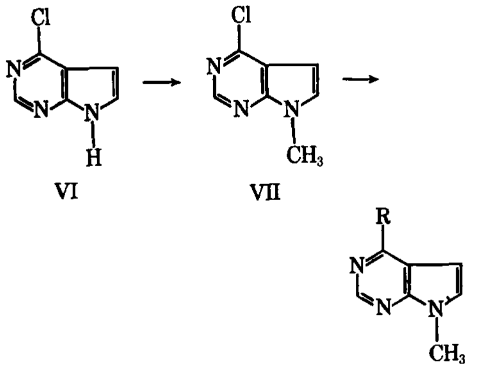
4-amino derivatives proceeds smoothly although requiring higher temperatures than the corresponding purines (6, 8). Hitchings *et al.* (10) reported the synthesis of various 4-alkylamino-7*H*-pyrrolo[2,3-*d*]pyrimidines by heating the 4-chloro starting material at elevated temperatures with an alkylamine. The facility by which the 4-chloro group of pyrrolo[2,3-*d*]pyrimidines serves as a leaving group for a variety of nucleophilic substitutions is therefore well established (8).

Two methods have been employed for the synthesis of VIII*a*–VIII*f*. The first method (Method A), which is similar to the method employed by Hitchings (10), produced low yields of product. The second method (Method B) whereby the volume of solvent was reduced and the acid-base steps were eliminated increased the yield of VIII*b* from 32 to 50%. Method B was therefore adopted for the synthesis of VIII*c*–VIII*f*. See Table I.

The ultraviolet spectra of the secondary amines (VIII*a*–VIII*d*) have maximums at 274 mμ (ε 11,840–13,120) and a more intense maximum at 232–233 mμ (ε 18,580–20,140) at pH 1, while in ethanol or at pH 13, only a broad absorption band at 273–278 mμ (ε 9,750–13,310) is observed. The spectra of the two tertiary amines, VIII*e* and VIII*f*, undergo a bathochromic shift to 280–284 mμ and 237–238 mμ at pH 1, and a shift to 281–290 mμ in ethanol or at pH 13 with the lower maximum having the more intense absorption. These spectra are characteristic of 4-amino and 4-propylamino-7*H*-pyrrolo[2,3-*d*]pyrimidines (6, 8, 10) as well as the corresponding purines (11). See Table II.

Representative proton magnetic resonance spectra were obtained on two of the free bases (VIII*c* and VIII*d*) and a HCl salt (VIII*a*). The pyrrole ring protons (C_5-H, C_6-H) both have coupling constants ($J_{5,6}$) = 3.5 c.p.s. The chemical shift (τ) of the 7-NCH_3 is 6.2–6.28 p.p.m. The chemical shift of the C_5-H is 2.82–3.12 p.p.m. and for the C_6-H, 3.38–3.66 p.p.m. The chemical shift of the pyrimidine ring C_2-H is further downfield at 1.65–1.93 p.p.m.

Representative pKa values range from 5.28 to 5.66 and are generally more basic than the corresponding 4-amino-7-methyl compound (pKa 5.02) (8), and tubercidin (pKa 5.3) (12). These pKa's are all favorable for producing high ratios of unionized to ionized forms of the drug at the pH of body fluids (7.4), and one would predict a high intracellular level of these drugs provided that they have high partition coefficients.



VI VII

VIII*a*, R = NHC_2H_5
VIII*b*, R = NHC_3H_7-*n*
VIII*c*, R = NHC_5H_{11}-*n*
VIII*d*, R = NHC_6H_{13}-*n*
VIII*e*, R = NC_4H_8
VIII*f*, R = NC_5H_{10}

TABLE III—CELL CULTURE CYTOTOXICITY

Compound	ED_{50} (mcg./ml.)[a]
VIII*a*[b]	25
VIII*b*[b]	27
VIII*c*[b]	47
VIII*d*[c]	56
VIII*e*[b]	42
VIII*f*[b]	100
VII[c]	>100
4-Amino-7-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidine (8)[c]	>100
4-Mercapto-7-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidine (8)[c]	>100
4-Chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine (7) (VI)[c]	26
4-Mercapto-7*H*-pyrrolo[2,3-*d*]pyrimidine (7) (IV)[c]	>100
4-Benzylthio-7*H*-pyrrolo[2,3-*d*]pyrimidine (7) (V)[c]	13
4-Methoxy-7*H*-pyrrolo[2,3-*d*]pyrimidine (7)[c]	28

[a] The dose (mcg./ml.) that inhibits growth of cells to 50% of control growth. [b] HCl salt. [c] Free base.

Synthesis of compounds VIII*a* and VIII*c* have previously been reported (10). The m.p. reported in the literature for VIII*a* agrees with the m.p. of the compound prepared in this laboratory. However, the m.p. previously reported for VIII*c* is in disagreement with that of the compound prepared in this laboratory. The structural assignments for VI and VII, intermediates in the synthesis of VIII*c*, are well established (8, 9). Reaction of VII with *n*-pentylamine to form VIII*c* as reported here is supported by comparison of the UV spectrum of VII (8) and Compounds VIII*a*, VIII*b*, and VIII*d* to the spectrum of VIII*c*. The bathochromic shift from 229 mμ (ε 23,050) and 272 mμ (ε 4,550) of VII (8) to 233 mμ (ε 20,140) and 274 mμ (ε 12,940) at pH 1, similar shifts at other pH's, plus similarity of the UV spectrum of VIII*c* to VIII*a*, VIII*b*, and VIII*d* indicate 4-alkylamino substitution. In addition, the C, H, N analysis and NMR of the free base (VIII*c*) and pKa data further support this. See Tables I and II. Based on this data, VIII*c* is considered to be the 4-*n*-pentylamino-7-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidine structure with physical properties as reported in Tables I and II.

Cytotoxicity studies have been conducted by the Cancer Chemotherapy National Service Center. The results obtained from several pyrrolo[2,3-*d*]-pyrimidine derivatives, synthesized and reported in previous papers (7, 8) and compounds VIII*a*-VIII*f* are given in Table III.

The compounds were all inactive although derivatives substituted with 4-alkylamino substituents (VIII*a*-VIII*e*) and 4-benzylthiopyrrolo[2,3-*d*]pyrimidine (7) (V) were all more active than the unsubstituted 4-amino-7-methyl- (8) and 4-mercaptopyrrolo-[2,3-*d*]pyrimidines (7) (IV), respectively. Conceivably, a bulky group on the 7 position, such as a cyclopentyl or cyclohexyl group, in addition to a 4-alkylamino- or 4-benzylthio- group, would increase activity.

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Keyphrases

Tubercidin analogs
Pyrrolo[2,3-*d*]pyrimidines—synthesis
pKa values—titration
TLC—separation
Proton magnetic resonance—identity
UV spectrophotometry—identity
NMR spectroscopy—identity
Cytotoxicity screening—tubercidin analogs

Alkaloids of *Peumus boldus.* Isolation of Laurotetanine and Laurolitsine

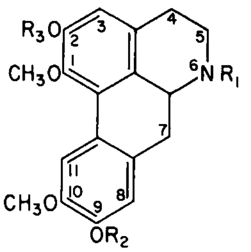
By D. W. HUGHES, K. GENEST, and W. SKAKUM

Two alkaloids, in addition to the six previously described, have been isolated from the leaves of *Peumus boldus* Molina. They were characterized by chromatographic and spectral data and shown to be the noraporphines, laurotetanine and laurolitsine.

DURING A chromatographic study of the alkaloids of the leaves of the South American shrub *Peumus boldus* Molina (*Monimiaceae*) (boldo leaves), the authors detected the presence of at least 17 alkaloids in a nonquaternary fraction obtained by chloroform extraction of the plant material after treatment with ammonia (1). The alkaloids were numbered 1–17 according to increasing R_f on thin-layer chromatography (TLC), and two were isolated and characterized as reticuline and the aporphine, isoboldine. Earlier, the aporphines boldine (I) (2), *N*-methyllaurotetanine (II), and isocorydine and the noraporphine, norisocorydine had been isolated (3). On the basis of paper chromatographic evidence the presence of sparteine has also been suggested (4).

This paper describes the isolation from boldo leaves and the characterization of two closely related noraporphines, laurotetanine (Alkaloid 6) (III) and laurolitsine (norboldine; Alkaloid 2) (IV). TLC examination of the crude alkaloid extract indicates that the leaves contain 0.005–0.007% laurotetanine and approximately 0.001% laurolitsine.

These alkaloids have been isolated from a number of species, particularly in the family *Lauraceae* (5, 6), sometimes together with boldine (5). A recent report describes the isolation of laurotetanine and *N*-methyllaurotetanine from another *Monimiaceae: Palmeria fengeriana* Perk (7).



I, $R_1 = CH_3$; $R_2 = R_3 = H$
II, $R_1 = R_3 = CH_3$; $R_2 = H$
III, $R_1 = R_2 = H$; $R_3 = CH_3$
IV, $R_1 = R_2 = R_3 = H$

EXPERIMENTAL

Isolation of Alkaloid 2—Alkaloids were extracted from 5 kg. boldo leaves and fractionated as described earlier (1) into four solutions (i, ii, iii, and iv). TLC examination of Solution i showed the presence of three main alkaloids (Nos. 1, 2, and 5). These were partially separated by column chromatography on silicic acid using chloroform–methanol mixtures as eluant. Fractions containing Alkaloid 2 were further resolved, first by rechromatography on silicic acid using benzene–methanol mixtures and then by preparative TLC. Alkaloid 2 was then found to be homogeneous by TLC, and was precipitated as an amorphous light brown solid with cyclohexane from ethanol solution. Yield 20 mg. (0.0004%); m.p. 128–134°.[1] Spectroscopic data

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[1] Melting points were taken on a Kofler melting point apparatus and are uncorrected.