Norit, and evaporated to dryness to give a yellow oil which redissolved in warm ethanol. The crystals that formed were collected by filtration, washed with ethanol, and dried *in vacuo* to give essentially pure product, yield 950 mg (34%), mp 121°. Thin layer chromatography on silica gel H (Merck) using $CHCl_3$–ethyl acetate (4:1) as the eluent showed 2,6-dichloropurine as the only contaminant. Recrystallization of a sample of the isolated material from boiling ethanol gave the pure product: mp 123°; λ_{max} [in mμ ($\epsilon \times 10^{-3}$)], pH 1, 7—252 (7.3), 273 (13.2), 280 (sh), pH 13—255 (sh), 258 (15.0), 265 (sh), 280 (sh); $\bar{\nu}_{max}$ (in cm^{-1}), 3115, 3060, 3050–3000 (CH), 1755, 1740, 1725 (C=O), 1595, 1560 (C=C, C=N), 1240, 1205 (COC).

Anal. Calcd for $C_{17}H_{18}Cl_2N_4O_7$: C, 44.37; H, 3.94; N, 12.18. Found: C, 44.25; H, 3.99; N, 12.12.

6-Chloro-9-(2,3,6-tri-*O*-acetyl-5-deoxy-β-D-*ribo*-hexofuranosyl)purine (IX).—A mixture of 6-chloropurine (1.5 g, 9.7 mmoles) and 1,2,3,6-tetra-*O*-acetyl-5-deoxy-β-D-*ribo*-hexofuranose (III, 3.4 g, 10.0 mmoles) was fused *in vacuo* (25 mm) at 130° with *p*-toluenesulfonic acid catalyst (75 mg) for 25 min. The resulting dark melt was cooled to room temperature, dissolved in $CHCl_3$ (10 ml), and filtered to remove unreacted 6-chloropurine. The filtrate was washed ($NaHC_3O_3$, water), dried ($MgSO_4$), and evaporated to dryness. The residue was triturated with ethanol and filtered to remove additional 6-chloropurine, and the filtrate was decolorized with Norit before it was evaporated to dryness *in vacuo*. Petroleum ether extraction of this residue partially removed the blocked sugar contaminant from the insoluble oily product, which was then dried *in vacuo*; yield 2.0 g (46%). Thin layer chromatography on silica gel H (Merck) using $CHCl_3$–ethyl acetate (3:1) indicated the material was suitable for use as an intermediate.

Acknowledgment.—The authors are indebted to Dr. W. J. Barrett and members of the Analytical Section of Southern Research Institute who performed the spectral and analytical determinations, to Dr. W. C. Coburn, Jr., and Mrs. M. Thorpe for their interpretation of the proton magnetic resonance spectra, and to Miss E. A. Dulmage for the cytotoxicity data reported herein.

Preparation and Antitumor Activity of Olivacine and Some New Analogs[1]

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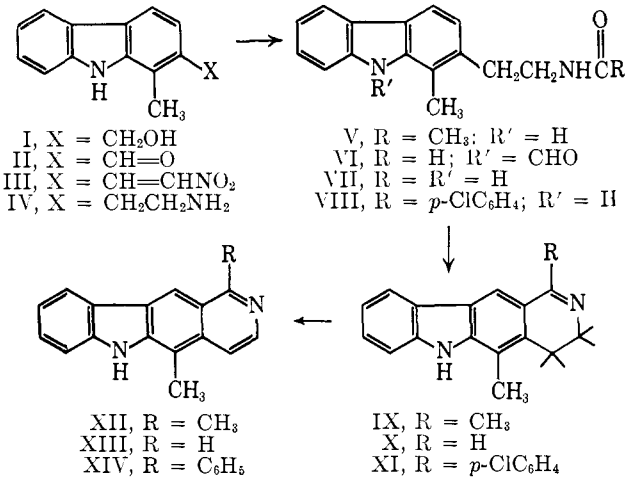
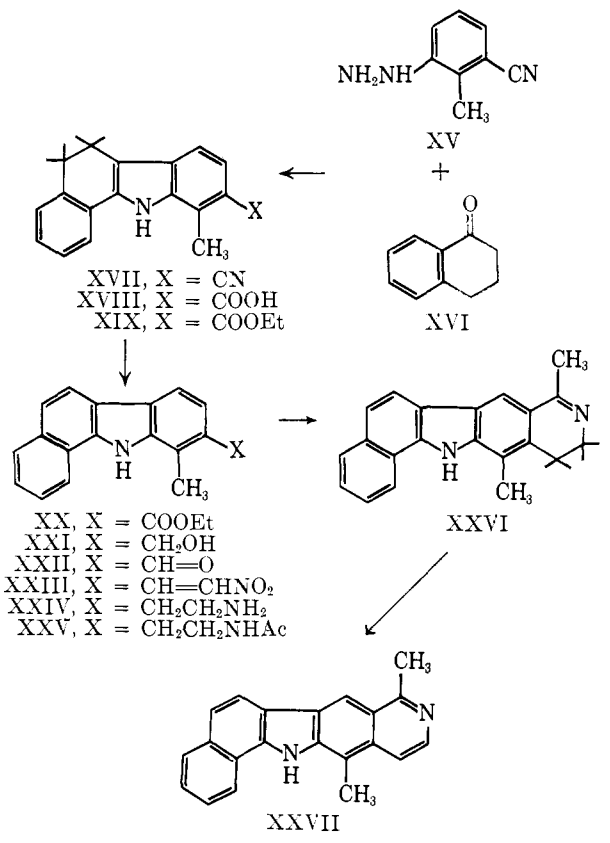
Received September 7, 1965

Preliminary reports from the Cancer Chemotherapy National Service Center[1] of potentially useful antitumor activity with the alkaloid olivacine (XII) necessitated the synthesis of large quantities for further biological testing. This has been accomplished (Scheme I) by revising a previous synthesis[2] to reduce the number of steps and avoid the use of diazomethane. The two previous syntheses[2,3] were useful mainly for the small amounts required for structure confirmation of XII. Structural requirements for activity in substituted pyridocarbazoles were studied briefly by the preparation of several analogs of XII. A demethyl derivative XIII of olivacine was easily accessible by Scheme I; this compound (XIII) is also a demethyl derivative of the alkaloid ellipticine (the 5,11-dimethylpyridocarbazole isomeric with olivacine). A recent synthesis[4] of ellipticine, but in very low yield, is similar in outline to the sequence in Scheme I which is convenient for quantities of XII and XIII. Preparation of a *p*-chlorophenyl derivative of olivacine was undertaken, because of the often encountered activity enhancement with this moiety, but the chlorine was lost in the final dehydrogenation and a simple phenyl derivative XIV resulted. A similar sequence (Scheme II) was used to prepare the benzoolivacine XXVII.

SCHEME I



SCHEME II



Biological Data.[5]—On the basis of incomplete testing results, the alkaloids related to olivacine and the corresponding dihydro compounds seemed to be poten-

(1) This work was carried out under the auspices of the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Public Health Service, Contract No. PH-43-64-500. The opinions expressed in this paper are those of the authors and not necessarily those of the Cancer Chemotherapy National Service Center.

(2) J. Schmutz and H. Wittwer, *Helv. Chim. Acta*, **43**, 793 (1960).

(3) E. Wenkert and K. G. Dave, *J. Am. Chem. Soc.*, **84**, 94 (1962).

(4) T. R. Govindachari, S. Rajappa, and V. Sudarsanam, *Indian J. Chem.*, **1**, 247 (1963).

(5) The compounds were screened under the auspices of the Cancer Chemotherapy National Service Center according to its protocols, outlined in *Cancer Chemotherapy Rept.*, **25**, 1 (1962).

tially active classes of compounds. Both olivacine (XII) and demethylolivacine (XIII) were active against leukemia L1210 transplanted in mice, as shown in Table I, but the phenyl compound XIV and benzoolivacine (XXVII) were inactive. Olivacine, in addition, was found to be active by cell culture methods against KB cells, with an ED_{50} of 0.4 μg/ml. The dihydro compounds X, XI, and XXVI were active against cell culture KB, with an ED_{50} consistently below 2 μg/ml, although dihydroolivacine IX showed borderline activity, ED_{50} = 2–7 μg/ml, in this system. The other carbazoles prepared exhibited no activity: compounds I through V were tested against leukemia L1210 and Sarcoma 180 transplanted in mice and against cell culture KB; compounds VIII, XX, and XXII–XXV were tested against KB cells.

TABLE I

TESTING *vs.* L1210 IN MICE[a]

Compd	Dose, mg/kg	Test/Control[b]
XII	84.0	1.41
	42.0	1.41
	21.0	1.35, 1.38
	10.0	1.32, 1.28
	5.00	1.14
	2.50	1.10
XIII	140	0.96, 1.41
	70.0	1.52, 2.21
	35.0	1.29, 1.70
	17.5	1.19, 1.41

[a] Six test mice and 6 control mice at each dose level. [b] Ratio of survival times.

Experimental Section[6]

Paper Chromatography of the olivacine compounds was studied by the descending technique in 1-butanol–methyl ethyl ketone–water (5:2:3) on Whatman No. 1 paper, with 25-μg samples to minimize streaking. Yellow spots were obtained; all exhibited bright yellow fluorescence ringed with white when observed under ultraviolet light. The R_f for olivacine (XII) was 0.85; demethylolivacine (XIII), 0.86; the phenyl compound XIV, 0.90; benzoolivacine (XXVII), 0.83. The corresponding dihydro compounds traveled slightly more slowly and exhibited dull yellow fluorescence under ultraviolet light; the partial separation from the pyridocarbazoles would not permit resolution of mixtures.

1-Methylcarbazole-2-carboxaldehyde (II).—To a suspension of 40.3 g (157 mmoles) of bis(pyridine)chromium oxide[7] in 400 ml of dry pyridine was added a solution of 16.6 g (78.5 mmoles) of 1-methyl-2-hydroxymethylcarbazole[2] (I) in 50 ml of pyridine. The mixture was stored at 25° overnight in a stoppered flask, then poured into 500 ml of water and extracted with 200 ml of ether. The aqueous layer was clarified of inorganic solids by filtration and extracted with three more portions of ether. The combined ether extracts were washed twice with 50-ml portions of water, four times with 50-ml portions of 3 *M* HCl, and twice with 50-ml portions of water. Concentration of the dried ether solution afforded 15 g of residual yellow solid. Brown impurities were removed by trituration with 100 ml of ether and filtration; 11.0 g (67%) of undissolved yellow aldehyde was collected on the filter, mp 156–164°. After several recrystallizations from CCl_4 the mp was 164–165.5° (lit.[3] 156–158°); λ_{max}^{EtOH} [mμ ($\epsilon \times 10^{-3}$)], 207 (18.5), 252 (39.0), 279 (7.25), 322 (24.0); λ_{max}^{Nujol} (μ), 3.03 (NH), 3.65 and 3.72 (aldehyde CH), 5.98 (C=O). An additional 2.3 g of aldehyde could be recovered from the ether filtrate after trituration and by further extraction of the aqueous pyridine layer.

1-Methyl-2-(2-nitrovinyl)carbazole (III) was obtained from 0.490 g (2.34 mmoles) of aldehyde II, 0.10 g of ammonium acetate, and 2.0 ml of nitromethane by the procedure of Whittle and Young.[8] The aldehyde at first dissolved and shortly thereafter a crystalline orange product separated. Ethanol recrystallization (250 ml/g) afforded 80% of III: mp 244–247°; λ_{max}^{EtOH} [mμ ($\epsilon \times 10^{-3}$)], 211 (20.1), 239 sh, 249 (31.4), 295 (7.22), 380 (24.9); CO absorption at 5.98 μ due to II was absent from the infrared spectrum; λ_{max}^{Nujol} (μ), 3.0 (NH), 7.62 (NO_2), 10.2 (unassigned), 10.45 (*trans* HC=CH).

Anal. Calcd for $C_{15}H_{12}N_2O_2$: C, 71.4; H, 4.80; N, 11.1. Found: C, 71.4; H, 5.07; N, 11.5.

1-Methyl-2-(2-aminoethyl)carbazole (IV).—A solution of 2.31 g (9.16 mmoles) of III in 60 ml of tetrahydrofuran (THF) was added, over a 30-min period, to a stirred suspension of 2.9 g (76 mmoles) of $LiAlH_4$ in 50 ml of THF. The mixture was kept at room temperature by occasional ice cooling. Stirring was continued for an additional 20 min, and the hydride was then decomposed by dropwise addition of 30 ml of 50% aqueous THF, with ice cooling. The opaque white mixture was filtered, the gelatinous solids were washed several times with THF, and the combined filtrate and washings were concentrated to form 2.2 g of residual solid. A solution in 50 ml of 3 *M* acetic acid was clarified by filtration through Celite and treated with 3 *M* NaOH until pH 11–12 was attained. The resultant precipitate weighed 1.54 g (75%); mp 169–176° (lit.[2] mp 172–175°); λ_{max}^{EtOH} [mμ ($\epsilon \times 10^{-3}$)], 216 (25.1), 238 (45.8), 248 (32.4), 258 (20.0), 296 (17.7), 323 (4.05), 336 (3.36); the infrared spectrum was free of bands at 10.2 and 10.45 μ that were present in the spectrum of III; weak absorption at 6.2 μ was not due to nitro impurity.

Olivacine (XII) was obtained from IV in the three steps described by Schmutz and Wittwer,[2] except that the final dehydrogenation was conducted with 30% palladium–carbon (5 or 10% palladium gave less pure product): mp 290–308° dec, Kofler 306–312° (lit.[2,3] 318–326° dec, 315–320°); λ_{max}^{EtOH} [mμ ($\epsilon \times 10^{-3}$)], 224 (24.5), 238 (20.4), 267 (36.8), 276 (53.2), 286 (78.5), 292 (74.9), 314 (4.45), 328 (5.67[9]), 343 (3.79); λ_{max} (0.01 *M* HCl in 85% EtOH) [mμ ($\epsilon \times 10^{-3}$)], 242 (29.9), 306 (80.4), 348 (6.55). The deviation from the literature melting point is not regarded as significant: ultraviolet extinctions indicate the compound is at least as pure as the previous synthetic sample.[2] The infrared spectrum in Nujol mull closely resembled the published curve obtained in KBr: λ_{max}^{Nujol} (μ), 6.18, 6.23, 6.62, 7.05, 7.45, 7.95, 8.15, 11.47, 12.26, 13.44.

1-Methyl-2-(2-formamidoethyl)-9-formylcarbazole (VI).—Formic acetic anhydride was prepared by heating a mixture of 4.20 ml (43.2 mmoles) of acetic anhydride and 1.68 ml (45.2 mmoles) of 97–100% formic acid at 50–60° for 20 min. A mixture of the anhydride at room temperature with 4.48 g (20.0 mmoles) of amine IV was heated at 50–60° for 2 hr and then concentrated *in vacuo*. The sticky residue was triturated with water until the washings were neutral. The damp residue was used directly in the next step, though in a separate experiment it could be dried to a powder (5.42 g, mp 60–125°) and crystallized from acetone–petroleum ether (bp 30–60°) to yield 1.06 g (19%), mp 147–158°. Infrared CO bands at both 5.89 and 6.03 μ and a single NH band at 3.06 μ indicated a diformyl derivative. After a second recrystallization, the mp was 160–162°; λ_{max}^{EtOH} [mμ ($\epsilon \times 10^{-3}$)], 229 (41.4), 235 sh (37.8), 249 (18.4), 260 (18.1), 270 (16.2), 283 (15.1), 295 sh (11.3), 312 (5.98), 325 (1.51), 337 (1.21). The nmr spectrum of a dilute but saturated solution qualitatively resembled that of the monoformyl compound VII below, except for presence of a singlet at τ 0.1, assigned to the carbazole NCHO, and for shift of the $ArCH_3$ to τ 7.33; similarity of the $-CH_2CH_2NHC(=O)$ pattern with that

(6) Corrected melting points were observed on a Fisher-Johns hot stage, or, when designated, with a Kofler micro hot stage under a polarizing microscope. Melting points above 200° often varied with rate and duration of heating, and were not reliable criteria of purity. Ultraviolet spectra best characterized the pyridocarbazoles, the dihydro derivatives, and the corresponding hydrochlorides, and provided quantitative determination of purity. These spectra were obtained with a Cary Model 11 recording spectrophotometer; shoulders are abbreviated sh. Infrared spectra were determined in Nujol mull; only strong bands or those significant for their assignment to functional groups are reported. The nmr spectra were determined with a Varian A-60 spectrometer, using chloroform-*d* solutions containing 4% tetramethylsilane as internal standard. Signals were observed as singlets unless otherwise designated as doublet (d), triplet (t), or quartet (q); chemical shifts are measured from multiplet centers. Concentration of solutions was done *in vacuo*. Many of the compounds showed strong affinity for trace amounts of solvent, as often was found in elemental analyses even after prolonged drying at 100° *in vacuo*.

(7) Eastman Organic Chemicals.

(8) E. A. Whittle and E. G. P. Young, *J. Med. Chem.*, **6**, 378 (1963).

(9) The extinction of 54,000 recorded at 330 mμ in ref 2 must be too large by a factor of 10.

in VII was regarded as evidence that the second formyl group was attached to the carbazole nitrogen atom, rather than to the side-chain nitrogen.

Anal. Calcd for $C_{17}H_{16}N_2O_2$: C, 72.8; H, 5.75; N, 9.99. Found: C, 73.1; H, 5.81; N, 10.22.

The mother liquors consisted of mixtures of VI with the monoformamide VII.

1-Methyl-2-(2-formamidoethyl)carbazole (VII).—A solution of the damp residual diformamide VI in 70 ml of warm methanol was treated with 10 ml of concentrated NH_4OH, stored for 1 hr at 25°, and concentrated. The residual product was used directly in the cyclization step, but alternatively could be recrystallized from 35 ml of acetone and 45 ml of Skellysolve B[10] to yield 3.32 g, mp 133–135°. After further recrystallization to give 2.68 g (53% yield), the infrared spectrum was unchanged, but the mp was 147–148.5°; λ_{max}^{EtOH} [$m\mu$ ($\epsilon \times 10^{-3}$)], 216 (25.3), 238 (43.6), 248 (32.3), 259 (20.3), 286 sh (10.8), 297 (17.5), 323 (4.39), 337 (3.50); the infrared spectrum showed two NH bands at 2.93 and 3.12 μ, and only a single CO, 5.99 μ; nmr data, τ 4.3 broad (side-chain NHC=O), 6.3 triplet of doublets (CH_2N), 7.0 t ($ArCH_2$), 7.52 ($ArCH_3$).

Anal. Calcd for $C_{16}H_{16}N_2O$: C, 76.2; H, 6.39; N, 11.1. Found: C, 76.1; H, 6.54; N, 11.2.

3,4-Dihydro-5-methyl-6H-pyrido[4,3-*b*]carbazole (X).—A stirred suspension of crude VII in 480 ml of toluene was heated to boiling, and the resultant solution was azeotropically dried by distillation of a little toluene and treated, at such a rate as to maintain a gentle reflux, with a mixture of 26.0 ml of $POCl_3$ and 26.0 ml of ethyl formate.[11,12] Solids gradually separated during 2 hr of further refluxing and stirring. The mixture was chilled to 5°, and the crude brown product (X·HCl) was collected on a filter. Purification was accomplished by trituration with 25 ml of hot 3 *M* HCl to dissolve the salt; the supernatant was filtered through Celite to remove brown tars. This trituration of the crude product was repeated once with hot acid, then several times with boiling water. Basification of the combined, cooled filtrates with concentrated NH_4OH caused separation of a gummy solid; the supernatant was decanted, and the solid was redissolved in 15 ml of warm 3 *M* HCl. This solution was diluted with 50 ml of water and basified with concentrated NH_4OH. The resultant fine yellow precipitate weighed 3.73 g (80% based on 4.48 g of amine IV), mp 240–273° dec, Kofler. Purity of about 90% was indicated by comparison of the ultraviolet spectrum with that of an analytical sample, mp 281–285° dec, Kofler, obtained in another experiment by acetone recrystallization; λ_{max}^{EtOH} [$m\mu$ ($\epsilon \times 10^{-3}$)], 238 (29.9), 248 (31.7), 276 (50.5), 292 sh (28.9), 316 (13.4), 328 (11.8), 342 (5.28); λ_{max} (0.01 *M* HCl in 85% EtOH) [$m\mu$ ($\epsilon \times 10^{-3}$)], 236 (22.4), 272 sh (26.2), 283 (45.1), 302 (13.9), 314 (19.4), 375 (23.0); λ_{max}^{Nujol} (μ), 6.11 and 6.20 (aryl C=C), 7.93, 8.10. 13.4.

Anal. Calcd for $C_{16}H_{14}N_2$: C, 82.0; H, 6.02; N, 12.0. Found: C, 82.0; H, 6.06; N, 12.1.

5-Methyl-6H-pyrido[4,3-*b*]carbazole (XIII, 1-Demethylolivacine).—A mixture of 4.66 g of X, 1.5 g of palladium black, and 300 ml of decalin was stirred and refluxed for 2 hr, chilled, and filtered to collect the solids, which were washed free of decalin with three 10-ml portions of benzene. The solids were extracted, in a slurry, with five 20-ml portions of hot dimethylformamide (DMF). The filtered extracts were combined, diluted with 100 ml of hot water, and cooled. The precipitated brown product (97–98% pure by ultraviolet data) was collected on a filter, washed with water, and redissolved in 250 ml of hot 1 *M* HCl. The solution was filtered to remove dark impurities and the orange filtrate was basified with concentrated NH_4OH and cooled to 5°. The pale yellow precipitate was collected and washed with water until the washings were neutral. The yield was 3.10 g (66%), mp 275–277° dec, Kofler; λ_{max}^{EtOH} [$m\mu$ ($\epsilon \times 10^{-3}$)], 224 (26.6), 238 (20.7), 273 (52.0), 284 (69.0), 293 (75.0), 313 (5.20), 328 (6.32), 373 (4.26), 390 (3.56);[13] λ_{max} (0.01 *M* HCl in 85% EtOH) [$m\mu$ ($\epsilon \times 10^{-3}$)], 238 (27.2), 246 sh (25.3), 271 sh (22.5), 306 (73.0), 350 (6.95); λ_{max}^{Nujol} (μ), 6.18 and 6.23 (aryl C=C), 8.07, 9.68, 12.28, 12.39, 13.33.

Anal. Calcd for $C_{16}H_{12}N_2$: C, 82.7; H, 5.21; N, 12.1. Found: C, 82.4; H, 5.46; N, 12.2.

1-Methyl-2-(2-*p*-chlorobenzamidoethyl)carbazole (VIII) was prepared from 8.96 g of IV and 8 ml of *p*-chlorobenzoyl chloride in 80 ml of pyridine at 50–60° for 30 min. A few drops of water was added and, 1 hr later, 500 ml of water; the product was collected (14.3 g, 99%) and recrystallized from 350 ml of acetone with 100 ml of benzene to yield 13.4 g (92%), mp 227–241°. An analytical sample, mp 232–241°, was obtained by recrystallization from DMF–water and acetone–benzene; the Kofler melting point was 239–241° with a phase change from amorphous to crystalline at *ca.* 220°; λ_{max}^{EtOH} [$m\mu$ ($\epsilon \times 10^{-3}$)], 217 (35.1), 239 (61.0), 248 (49.5), 258 (30.4), 286 sh (13.9), 296 (21.8), 323 (4.74), 336 (3.51); λ_{max}^{Nujol} (μ), 2.90 and 3.00 (NH), 6.08 (amide C=O), 6.44 (amide II).

Anal. Calcd for $C_{22}H_{19}ClN_2O$: C, 72.8; H, 5.28; Cl, 9.77; N, 7.72. Found: C, 72.9; H, 5.32; Cl, 10.0; N, 7.51.

1-(*p*-Chlorophenyl)-3,4-dihydro-5-methyl-6H-pyrido[4,3-*b*]carbazole (XI).—A mixture of 10.10 g (28 mmoles) of *p*-chlorobenzamide VIII, 1 l. of toluene, and 38 ml (445 mmoles) of $POCl_3$ was refluxed for 6 hr, then cooled to 5°, and the solid product was collected on a filter. This crude hydrochloride was insoluble in hot HCl. It was dissolved in 125 ml of DMF, and the solution diluted with 125 ml of water and made alkaline (pH 12) with concentrated NH_4OH to precipitate the free base. The cream-colored solid was collected and washed with water (in another experiment where the reaction time was 1 hr, 42% of starting material was recovered from the product at this point by extraction with boiling acetone), 8.95 g (92%), mp 309–315° dec, Kofler. A sample for analysis mp 315–317° dec, Kofler, was obtained by recrystallization from DMF–water; λ_{max}^{EtOH} [$m\mu$ ($\epsilon \times 10^{-3}$)], 240 (40.6), 248 (38.0), 283 (34.8), 298 sh (22.3), 320 (12.3), 331 (13.6), 343 sh (8.60); λ_{max} (0.01 *M* HCl in 85% EtOH) [$m\mu$ ($\epsilon \times 10^{-3}$)], 222 (20.9), 232 (22.3), 238 (23.3), 245 (23.5), 278 sh (25.8), 287 (31.2), 306 (13.2), 319 (14.0), 392 (20.0).

Anal. Calcd for $C_{22}H_{17}ClN_2$: C, 76.6; H, 4.97; Cl, 10.3; N, 8.13. Found: C, 76.2; H, 5.08; Cl, 10.3; N, 8.20.

1-Phenyl-5-methyl-6H-pyrido[4,3-*b*]carbazole (XIV).—Dehydrogenation of XI as described in preparing XIII, but with 30 or even 5% palladium–charcoal, resulted in simultaneous hydrogenolysis of as much as 90% of the aryl chloride. The remaining chlorine was removed by hydrogenation at 1 atm with 5% palladium–charcoal in DMF solution (1 g/20 ml) containing 1% triethylamine. After filtration to remove the catalyst, addition of 7 vol of water precipitated the product (about 97% pure by ultraviolet data), which was recrystallized from DMF–water (5:10 ml/g), mp 132–140° with solidification and remelting at 222–257° Kofler. The analytical sample was dried at 100° and 0.2 mm for several hours to remove solvated DMF (indicated by infrared amide absorption at 6.0 μ), mp 259–261° Kofler. A sample with identical spectra was alternatively obtained by deliberate initial hydrogenolysis of the chlorine in XI, then dehydrogenation, according to the above procedures, mp 257–259° Kofler; λ_{max}^{EtOH} [$m\mu$ ($\epsilon \times 10^{-3}$)], 225 (28.3), 243 (24.7), 290 (67.5), 295 (73.2), 333 (6.50), 348 (5.30), 379 (4.94), 395 (4.70); λ_{max} (0.01 *M* HCl in 85% EtOH) [$m\mu$ ($\epsilon \times 10^{-3}$)], 237 (29.0), 250 (25.2), 258 (25.5), 276 (28.1), 314 (85.2), 355 (9.16); λ_{max}^{Nujol} (μ), 3.1 (NH), 6.05–6.3 (aryl C=C, 3 sharp bands plus side bands), 7.95, 8.04, 11.28, 11.51, 12.19, 12.95, 13.35, 13.7; a second form was sometimes obtained, as seen by closer resemblance to the olivacine spectrum in the infrared (μ), 3.2 and 3.3 (NH), 6.13–6.28 (aryl, 2 sharp bands), an additional band at 11.28, and was apparently due to a different degree of association.

Anal. Calcd for $C_{22}H_{16}N_2$: C, 85.7; H, 5.23; N, 9.09. Found: C, 85.7; H, 5.13; N, 9.29.

10-Methyl-5,6-dihydro-11H-benzo[*a*]carbazole-9-carboxylic Acid (XVIII).—Borsche reaction between 3-hydrazino-2-methylbenzonitrile[2] (XV) and α-tetralone (XVI), as with cyclohexanone in the olivacine synthesis,[2] afforded 44% of crude nitrile XVII, mp 169–175°; λ_{max}^{EtOH} [$m\mu$ ($\epsilon \times 10^{-3}$)], 221 (19.7), 235 (23.0), 316 (19.3), 342 (15.0). This was saponified, without purification, in boiling ethylene glycol.[2] The crude acid (93%), obtained by neutralization, was suspended in water (4 ml/g) and converted back to the sodium salt (also in suspension; the salt was soluble at a dilution of 800 ml/g at pH 13) by stirring

(10) A hydrocarbon fraction, bp 62–70°.

(11) R. H. F. Manske and M. Kulka, *J. Am. Chem. Soc.*, **72**, 4998 (1950).

(12) G. B. Marini-Bettolo and J. Schmutz, *Helv. Chim. Acta*, **42**, 2146 (1959).

(13) Difficult solubility in neutral ethanol at 0.5 mg/100 ml was sometimes a problem in preparing solutions for spectra and could be circumvented by using ethanol containing an equivalent of acid, which was then neutralized; the data obtained in neutral solution provided the best quantitative assay of XIII.

with 20% NaOH (2.5 ml/g). The insoluble salt was collected and washed with water, acetone, and ether, removing pink color; acidification of an aqueous suspension regenerated the acid, which was collected and recrystallized from acetone–petroleum ether (1:1, 30 ml/g), 62% yield, mp 247–249°. An analytical sample melted at 259–265°, but was unchanged in the ultraviolet; λ_{max}^{EtOH} [mμ (ε × 10⁻³)], 220 (22.8), 254 (27.5), 259 (27.6), 331 (23.8), 350 (23.3); λ_{max}^{Nujol} (μ), 2.90 (NH), 3.7–3.9 (acid OH), no CN at 4.5, 5.95–6.0 (acid C=O), 6.19 (aryl), 7.6 and 7.8 (unassigned), 10.68 (COOH), 13.02 and 13.32 (aryl).

Anal. Calcd for $C_{18}H_{15}NO_2$: C, 78.0; H, 5.45; N, 5.05. Found: C, 78.2; H, 5.57; N, 5.20.

Ethyl 10-methyl-5,6-dihydro-11H-benzo[*a*]carbazole-9-carboxylate (XIX) was obtained by esterification of XVIII with ethanolic HCl and recrystallized from ethanol–petroleum ether (bp 30-60°) (1:2), 80% yield, mp 142-148°. Further recrystallization afforded the analytical sample: mp 145–146°; λ_{max}^{EtOH} [mμ (ε × 10⁻³)], 223 (21.8), 253 (28.0), 260 (28.3), 289 (9.50), 328 (22.1); λ_{max}^{Nujol} (μ), 3.0 (NH), 6.02 (ester C=O), no COOH at 3.7–3.9 or 10.7; nmr data, τ 1.45 broad (NH), 2.1–3.0 m (6 aryl H), 5.67 q (CH_2 of Et), 7.1 plus side peak ($ArCH_2CH_2Ar$), 7.27 ($ArCH_3$), 8.65 t (CH_3 of Et).

Anal. Calcd for $C_{20}H_{19}NO_2$: C, 78.7; H, 6.27; N, 4.59. Found: C, 78.5; H, 6.37; N, 4.57.

Ethyl 10-methyl-11H-benzo[*a*]carbazole-9-carboxylate (XX) was obtained from XIX by dehydrogenation[2] and recrystallization from ether–petroleum ether (bp 30–60°) (1:1), 60% yield; mp 170–174°; λ_{max}^{Nujol} (μ), 2.98 (NH), 5.92 (ester C=O), 7.91, 9.48, 12.51, 12.78, 13.02, 13.7 (unassigned). A purity of 95% was indicated by comparison of the ultraviolet spectrum with that of an analytical sample obtained by further recrystallization; mp 179–180°; λ_{max}^{EtOH} [mμ (ε × 10⁻³)], 237 (25.5), 253 (35.2), 288 (55.6), 313–318 broad (22.5); nmr data, τ 1.17 broad (NH), 1.78–2.55 m (8 aryl H), 5.56 q (CH_2 of Et), 7.1 ($ArCH_3$), 8.56 t (CH_3 of Et). The analytical sample exhibited a second crystal form in the infrared (μ), 3.0 (NH), 6.02 (ester C=O), 7.6, 9.75, 12.38, 12.65, 12.90, 13.5 (unassigned).

Anal. Calcd for $C_{20}H_{17}NO_2$: C, 79.2; H, 5.65; N, 4.62. Found: C, 79.0; H, 5.61; N, 4.63.

9-Hydroxymethyl-10-methyl-11H-benzo[*a*]carbazole (XXI).—The ester XX (1.76 g, 5.80 mmoles) in 150 ml of ether was treated slowly, to avoid excessive heating, with 0.80 g of $LiAlH_4$ suspended in 30 ml of ether. After being stirred for 1 hr, the mixture was decomposed with ethanol–ether (1:1) and then with 3 *M* HCl. The product was isolated from the ether layer and was recrystallized from ether–petroleum ether, 71% yield; mp 178–181°; λ_{max}^{EtOH} [mμ (ε × 10⁻³)], 238 sh, 246 (42.0), 254 (45.6), 281 (49.5), 298 sh (19.3), 308 (22.4), 322 (7.03), 338 (6.70), 354 (7.13); λ_{max}^{Nujol} (μ), 2.83 and 3.02 (OH and NH), 10.15, 12.45, 12.75, 13.02, 13.38, 13.82 (unassigned), no ester C=O near 6.0 μ.

Anal. Calcd for $C_{18}H_{15}NO$: C, 82.7; H, 5.79; N, 5.36. Found: C, 82.8; H, 6.00; N, 5.60.

In one experiment, a solid melting about 230° was also obtained in 25% yield, which appeared to be largely the 9,10-dimethyl compound by elemental analysis, and which was probably formed by hydrogenolysis of the benzylic alcohol.

10-Methyl-11H-benzo[*a*]carbazole-9-carboxaldehyde (XXII).—The alcohol XXI was oxidized as described in the preparation of II. The crude product (97% yield), mp 235–270°, was recrystallized from acetone–CCl_4 (1:25); mp 258–270°; λ_{max}^{EtOH} [mμ (ε × 10⁻³)], 208 (25.2), 232 (22.7), 254 (32.0), 298 (38.7), 336 (21.9); λ_{max}^{Nujol} (μ), 3.0 (NH), 3.63 (aldehyde CH), 5.93 and 6.0 (aldehyde C=O), 6.2 (aryl), 6.62, 7.58, 7.93, 9.16, 12.61, 12.81, 13.62 (unassigned). The bright yellow compound appeared to have a strong affinity for small amounts of solvents or moisture.

Anal. Calcd for $C_{18}H_{13}NO$: C, 83.4; H, 5.05; N, 5.40. Calcd for $C_{18}H_{13}NO \cdot 0.25H_2O$: C, 82.4; H, 5.13; N, 5.34. Found: C, 82.5; H, 4.90; N, 5.14. Found (after re-drying at 60° at 0.2 mm. for 20 hr.): C, 83.1; H, 5.25.

10-Methyl-9-(2-nitrovinyl)-11H-benzo[*a*]carbazole (XXIII).—A mixture of 7.5 g (0.029 mole) of XXII, 90 ml of nitromethane, and 1.5 g of ammonium acetate was heated on the steam bath for 1.5 hr. About 50 ml of the nitromethane was then removed *in vacuo* and the residual suspension was chilled. The red solid was collected and washed with ethanol (4.0 g, 46%, mp 240–260°), then was recrystallized from ethyl acetate–ethanol–benzene (1:1:1) to yield 3.6 g (41%); mp 255–260°; λ_{max}^{EtOH} [mμ (ε × 10⁻³)], 256 (27.1), 279 (21.9), 313 (10.8), 322 (10.7), strong broad absorption in the visible *ca.* 400 mμ; λ_{max}^{Nujol} (μ), 2.92 (NH), 6.22 (aryl), 7.62 (NO_2), 10.3 (unassigned), 10.5 (*trans*-CH=CH), 12.57 (aryl), no aldehyde carbonyl at 5.9–6.0.

Anal. Calcd for $C_{19}H_{14}N_2O$: C, 75.5; H, 4.67; N, 9.27. Found: C, 75.0; H, 4.78; N, 9.34.

10-Methyl-9-(2-aminoethyl)-11H-benzo[*a*]carbazole (XXIV).—A solution of 3.20 g (10.6 mmoles) of XXIII in 100 ml of anhydrous THF was added, during 20 min, with occasional cooling, to a stirred suspension of 4.50 g (118 mmoles) of $LiAlH_4$ in 60 ml of THF. After 40 min, the stirred mixture was treated with 50 ml of aqueous THF (1:1) to decompose the hydride. The gelatinous solids were removed by filtration and washed with THF. The combined filtrate and washings were concentrated to form a residual syrup which was dissolved in 15 ml of 3 *M* acetic acid; the solution was treated with Norit and filtered through Celite. Neutralization of the filtrate with 10% NaOH caused precipitation of the product, 2.33 g (80%), mp 92–100°. A sample for analysis was reprecipitated, mp 87–91°, and dried at 60° (0.2 mm) for 15 hr, but was unchanged in infrared absorption; λ_{max}^{Nujol} (μ), 2.91 and 3.0–3.15 (NH), 6.35 broad (aryl), 6.52 (unassigned), 12.5 (aryl, very strong); λ_{max}^{EtOH} [mμ (ε × 10⁻³)], 228 (26.8), 246 (37.8), 255 (44.0), 280 (44.6), 307 (21.9), 322 sh (7.88), 337 (7.10), 353 (7.15).

Anal. Calcd for $C_{19}H_{18}N_2 \cdot 0.5H_2O$: C, 80.5; H, 6.76; N, 9.88. Found: C, 80.8; H, 6.29; N, 10.0.

In some cases, a product melting at 150–160° was obtained, which was identical in spectra and gave an acceptable analysis.

Anal. Found: C, 80.0; H, 6.34; N, 9.68; 0.7% ash.

10-Methyl-9-(2-acetamidoethyl)-11H-benzo[*a*]carbazole (XXV) was obtained by acetylation[2] of XXIV, and the crude residual solid was recrystallized from chloroform–Skellysolve B[10] (40:50 ml/g). The brownish solid that initially separated (mp below 180°, 10% yield calcd as product) was discarded and the second crop was collected as the product (65%), mp 208–218° dec. A sample again recrystallized melted at 216–220° dec; λ_{max}^{EtOH} [mμ (ε × 10⁻³)], 229 (29.0), 246 (40.6), 255 (46.2), 280 (47.1), 307 (22.4), 322 sh (7.56), 337 (6.88), 353 (6.95); λ_{max}^{Nujol} (μ), 2.93 (indole NH), 3.02 (amide NH), 6.01 (amide C=O), 6.52 (amide II), 12.40 (aryl).

Anal. Calcd for $C_{21}H_{20}N_2O$: C, 79.7; H, 6.37; N, 8.85. Calcd for $C_{21}H_{20}N_2O \cdot 0.1CHCl_3$: C, 77.2; H, 6.17; Cl, 3.24; N, 8.53. Found: C, 77.3; H, 5.99; Cl, 3.14; N, 8.46.

10,11-Dihydro-8,12-dimethyl-13H-benzo[*a*]pyrido[3,4-*h*]carbazole (XXVI).—The amide XXV was cyclized as described in the preparation of XI. The resultant yellow hydrochloride was washed with benzene and with ethanol and suspended in ethanol (30 ml/g) and basified with 15 *M* NH_4OH. The resultant free base partly dissolved, but was quite insoluble after adding an equal volume of water, and was collected as a pale tan solid (free of distinct infrared maxima exhibited by the salt at 3.22, 3.60, 3.68, and 6.07 μ), 3.50 g, 85% yield. The purity was 85% by comparison of the ultraviolet spectrum with that of an analytical sample (50% yield) which was precipitated from a DMF solution by adding water, then triturated with ethanol (30 ml/g). The compound decomposed when heated above 280°; λ_{max}^{EtOH} [mμ (ε × 10⁻³)], 231 (21.9), 249 (16.5), 255 sh (19.2), 279 (77.1), 313 sh (24.6), 328 sh (17.4), 353 (6.35); λ_{max} (0.01 *M* HCl in 85% EtOH) [mμ (ε × 10⁻³)], 226 (19.4), 239 (17.2), 246 (15.6), 273 (69.4), 288 sh (27.2), 308 (19.8), 319 (24.7), 332 (42.0), 347 (19.4), 366 (17.3); λ_{max}^{Nujol} (μ), 6.15 and 6.19 (aryl), 8.0, 10.98, 11.3 (unassigned), 12.35 (aryl).

Anal. Calcd for $C_{21}H_{18}N_2$: C, 84.5; H, 6.08; N, 9.39. Found: C, 84.4; H, 6.08; N, 9.56.

8,12-Dimethyl-13H-benzo[*a*]pyrido[3,4-*h*]carbazole (XXVII).—Dehydrogenation of XXVI, as described in preparing XIII, but with an equal weight of 30% palladium–carbon, afforded 80% of crude product, which was dissolved in hot 3 *M* acetic acid (70 ml/g; 15% by wt was insoluble) and reprecipitated with base. Finally, recrystallization from DMF–water (1:1, 50 ml/g) gave 34% of XXVII, which was dried at 140° (0.2 mm) for 20 hr; λ_{max}^{EtOH} [mμ (ε × 10⁻³)], 233 (26.0), 238 sh (25.0), 283 (97.2), 290 sh (80.6), 314 (14.0), 326 (15.7), 337 (13.2), 352 (6.50), 382 (5.93), 397 (5.55); λ_{max} (0.01 *M* HCl in 85% EtOH) [mμ (ε × 10⁻³)], 233 (26.0), 246 (24.6), 264 sh, 274 (30.1), 294 sh, 309 (62.5), 330 (28.3), 351 (12.9); λ_{max}^{Nujol} (μ), 6.13, 6.26, 6.37 (weak, aryl), 6.51, 11.42 (unassigned), 12.48 (strong, aryl), a weak band at 6.0 μ indicated the solvated DMF that was found on elemental analysis. The compound decomposed upon heating above 330°.

Anal. Calcd for $C_{21}H_{16}N_2$: C, 85.1; H, 5.44; N, 9.45. Calcd for $C_{21}H_{16}N_2 \cdot 0.05C_3H_7NO$: C, 84.7; H, 5.50; N, 9.57. Found: C, 84.7; H, 5.65; N, 9.84.

Acknowledgments.—The authors are indebted to Dr. Peter Lim for the infrared interpretations and to his staff for the spectral data and paper chromatography. The authors also thank Dr. Lois Durham of Stanford University for some of the nmr interpretations.

Compounds Derived from L-Threitol 1,4-Bismethanesulfonate

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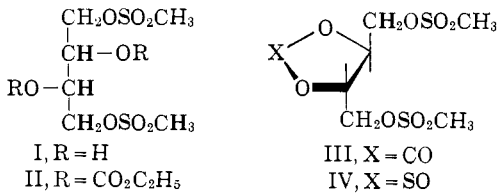
Received September 18, 1965

In continuation of the synthesis of the 1,4-bismethanesulfonates of the stereoisomeric butanetetraols and related compounds,[1] and for reasons already discussed[1] we prepared the following derivatives of L-threitol 1,4-bismethanesulfonate (I) with the purpose of obtaining substances for investigation as anticancer agents, and of getting further information about the structure–activity relationship of this type of alkylating agents: di-2,3-*O*-carbethoxy-L-threitol 1,4-bismethanesulfonate (II), the 2,3-cyclic carbonate of I (III) [L-4,5-bismethylsulfonyloxymethyl-1,3-dioxolanone-(2)], and the 2,3-cyclic sulfite of I (IV) (L-4,5-bismethylsulfonyloxymethyl-1,3,2-dioxathiolane 2-oxide). The compounds were obtained by reaction of I with ethyl chloroformate, phosgene, and thionyl chloride, respectively.



Screening by the Cancer Chemotherapy National Service Center, National Institutes of Health, has revealed no significant difference in antineoplastic activity between these derivatives and that of I,[2] as far as the Walker carcinosarcoma 256 system is concerned. Moreover, in the KB cell culture system the ED_{50} determined for II is more than ten times as high as that of I, III, and IV. A summary of the test data is presented in Tables I and II.

TABLE I

SCREENING DATA IN THE WALKER CARCINOSARCOMA 256 (SUBCUTANEOUS) SYSTEM

Compd	Daily dose, mg/kg[a]	Survivors	Mean tumor weight test/control, %[b]	ED_{90},[c] mg/kg/day
II	200	6/6	0	
	100	6/6	6	120
	50	6/6	95	
	25	6/6	75	
III	200	6/6	0	
	100	6/6	47	130
	50	6/6	70	
	25	6/6	92	
IV	200	6/6	0	
	100	6/6	17	130
	50	6/6	48	
	25	6/6	105	

[a] Administered ip once daily, days 1 through 5 postinoculation. [b] Sacrificed and evaluated 10 days postinoculation. [c] The dose that inhibits growth to 10% of control growth.

TABLE II

SCREENING DATA IN THE KB CELL CULTURE SYSTEM

Compd	ED_{50},[a] μg/ml	Slope[b]
I[c]	5.0	−0.43
	11	−0.56
	3.6	−0.28
II	>100	
III	7.2	−0.54
IV	9.8	−0.55

[a] The dose that inhibits growth to 50% of control growth. [b] Change of response for each 1 log change of dose. [c] Tested at different days.

Experimental Section[3]

Di-2,3-*O*-carbethoxy-L-threitol 1,4-Bismethanesulfonate (II).—To a solution of L-threitol 1,4-bismethanesulfonate (10 g) in pyridine (50 ml), ethyl chloroformate (8 ml) was added dropwise while stirring at 0 to 5°. The mixture was stirred for additional 30 min, kept in a refrigerator for 40 hr, and then poured into 4 *N* HCl (100 ml) while cooling. The separated oil was extracted with chloroform, the organic layer was washed several times with water, and after drying ($MgSO_4$) the solvent was removed by evaporation *in vacuo*. The residue was triturated with diethyl ether, and the resulting crystalline material (12.7 g) was recrystallized from benzene–diethyl ether (decolorizing carbon) yielding II (10.2 g) with mp 58–61°. The analytically pure compound was reached by filtration of a solution of crude II in benzene through aluminum oxide (anionotropic, activity grade I), evaporating the filtrate *in vacuo*, and triturating the residue with a small amount of benzene, affording crystallization, followed by recrystallization from chloroform–diethyl ether, mp 62–64° (shrinking at 58°), $[\alpha]^{20}D$ −9.8° (*c* 2, acetone).

Anal. Calcd for $C_{12}H_{22}O_{12}S_2$: C, 34.12; H, 5.25; S, 15.18. Found: C, 34.17; H, 5.31; S, 15.15.

L-4,5-Bismethylsulfonyloxymethyl-1,3-dioxolanone-(2) (III).—To a solution of L-threitol 1,4-bismethanesulfonate (10 g) in pyridine (40 ml), a solution of phosgene (4.5 g) in diethyl ether (20 ml) was added dropwise while stirring at 0 to 5°. The reaction mixture was kept in a refrigerator for 70 hr and then poured into a mixture of 4 *N* HCl (100 ml) and ice. The solid (10.5 g) which separated was filtered off and recrystallized from acetone–diethyl ether (75 ml each) (decolorizing carbon), yielding crude III (7.9 g) with mp 123–125°. The analytically pure and colorless III was obtained by filtration of a warm solution of the crude material in acetone through aluminium oxide (anionotropic, activity grade I), and precipitation by adding diethyl ether to the filtrate, followed by recrystallization from a small amount of acetone, mp 126–127°, $[\alpha]^{20}D$ −62.6° (*c* 2, acetone).

Anal. Calcd for $C_7H_{12}O_9S_2$: S, 27.63; H, 3.98; S, 21.07. Found: C, 27.82; H, 4.19; S, 21.01.

L-4,5-Bismethylsulfonyloxymethyl-1,3,2-dioxathiolane 2-Oxide (IV).—A suspension of L-threitol 1,4-bismethanesulfonate (10 g) in $SOCl_2$ (25 ml) was refluxed for 45 min. After cooling, the obtained solution was evaporated to dryness *in vacuo*. The oily residue was treated with benzene (25 ml), and the evaporation was repeated. The crystalline residue was dissolved in ethyl acetate (200 ml); the solution was washed twice with water, dried ($MgSO_4$), and evaporated *in vacuo* to yield IV (11.5 g) with mp 97.5–99°. The crude material was recrystallized from ethyl acetate (50 ml) and redissolved in warm ethyl acetate (75 ml); the warm solution was diluted with diethyl ether (50 ml) and chilled after filtration over decolorizing carbon removing turbidity, mp 98.5–100°, $[\alpha]^{20}D$ −105.5° (*c* 2, acetone).

(1) P. W. Feit, *J. Med. Chem.*, **7**, 14 (1964).

(2) F. R. White, *Cancer Chemotherapy Rept.*, **24**, 95 (1962).

(3) Analyses were by G. Cornali and W. Egger of these laboratories. Melting points were taken in open glass capillaries and rounded off to half degrees, using a Hershberg apparatus with thermometers subdivided in 0.1°. Technical assistance was by Th. Rolle.