two TLC systems: MeOH–$CHCl_3$ (1:1) and *n*-BuOH–AcOH–H_2O (6:3:1); R_f values were 0.79 and 0.72, respectively; ir λ_{max}^{KBr} 6.52 (1534) and 7.41 μ (1350 cm^{-1}) (CNO_2).

This method was used to prepare S^6 derivatives of **71** and **72** listed in Table VI.

Method B. N^6-(4-Nitrobenzyl)adenosine (11). A mixture of **73** (200 mg, 0.69 mmol), 4-nitrobenzylamine (320 mg, 2.10 mmol), and calcium carbonate (140 mg, 1.40 mmol) in EtOH (10 ml) was stirred at reflux temperature for 20 hr. The reaction mixture was filtered while hot and the filtrate evaporated in vacuo; the residue was dissolved in 5 ml of hot MeOH and, after standing at 10°, crystalline material formed which was washed with cold Et_2O and dried (yield 196 mg (70%), mp 176°). The product showed one spot on TLC in MeOH–$CHCl_3$ (1:9); R_f 0.66; ir λ_{max}^{KBr} 6.51 (1536) and 7.40 μ (1351 cm^{-1}) (CNO_2).

This method was also used to prepare **6** and **10** (Table VI).

Method C. O^6-Benzylinosine (14). Cleaned sodium metal (116 mg, 5.0 mmol) dissolved in dry benzyl alcohol (5 ml) was added to **73** (420 mg, 1.4 mmol) and heated at 85° with stirring for 1 hr. The reaction mixture was evaporated to dryness in vacuo at 60–70° and the residue dissolved in 5 ml of H_2O; this solution was adjusted to pH 6 with AcOH and extracted several times with EtOAc. The combined EtOAc extracts were evaporated to a small volume and treated with charcoal; upon the addition of petroleum ether, crystalline material formed at room temperature and was washed with Et_2O, dried, and recrystallized from EtOAc–petroleum ether [yield 480 mg (91.5%), mp 173°]. The product showed one spot on TLC in MeOH–$CHCl_3$ (1:9); R_f 0.60; ir λ_{max}^{KBr} 6.19 (1616) and 7.35 μ (1361 cm^{-1}).

Physical properties and chromatographic data for these and related compounds synthesized by the above methods are given in Tables VI–VIII.

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Synthesis and Cell Culture Studies on the Antiviral Activity of 5-Mercaptomethyl-2'-deoxyuridine†

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Treatment of 5-mercaptomethyluracil (I) with trimethylsilyl chloride in the presence of triethylamine gave 2,4,5-tris-(trimethylsilyl)-5-mercaptomethyluracil (II) which, upon coupling with 2-deoxy-3,5-di-*O*-(*p*-toluoyl)-D-*erythro*-pentofuranosyl chloride, furnished an anomeric mixture of fully substituted 2'-deoxy ribonucleosides. The nucleoside with β configuration (III) was predominantly formed and was isolated as a crystalline solid. The free nucleoside IV was obtained by removal of blocking groups by sodium methoxide catalyzed deacylation, deionization under reducing atmosphere, and chromatography on neutral alumina. IV is oxidized to the corresponding disulfide V in solution in the absence of thiols. IV was found to be markedly inhibitory against the herpes virus of infectious bovine rhinotracheitis (IBR). Against this virus, IV was found to be as potent as 5-iododeoxyuridine and cytosine arabinoside when added 18 hr before virus infection.

5-Hydroxymethylpyrimidines are unique components of the DNA of a virulent group of *Bacillus subtilis*[1-3] and *Escherichia coli* T-even bacteriophages.[4] 5-Substituted pyrimidines have also been postulated as intermediates during the conversion of thymine to RNA pyrimidines.[5] Antimetabolites of 5-hydroxymethyl-2'-deoxyuridine ($OH-CH_2UdR$) may show selective toxicity toward viruses if

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Table I. Relative in Vitro Antiviral Activity of IV,[a] V, IUdR, and Ara-C against IBR Virus[b] in Secondary BFK Cells

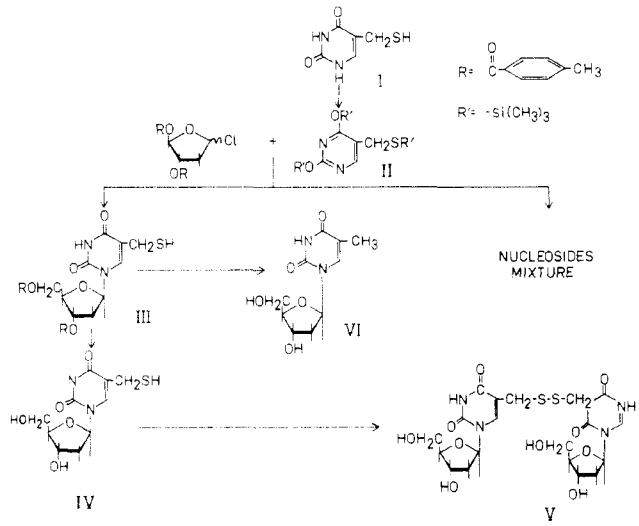
	Log reduction in virus titer after treatment at times					
	18 hr preinfection		15 min postinfection		18 hr postinfection	
Compd added	2.8 μg/ml	280 μg/ml	2.8 μg/ml	280 μg/ml	2.8 μg/ml	280 μg/ml
None (control)	0	0	0	0	0	0
GSH	0	0	0	0	0	0
IV	1.0	2.0	0.5	1.0	0	0.5
V	0	0.5	0	0	0	0
IUdR	1.0	2.0	1.0	2.0	0	1.0
Ara-C	0.5	2.5	0.5	2.0	0.5	1.5

[a] 1-(2-Deoxy-D-*erythro*-pentofuranosyl)-5-mercaptomethyluracil. [b] 100 $TCID_{50}$/ml.

they are preferentially incorporated into viral DNA. The sulfur analogs of $OHCH_2UdR$ may afford a comparison of the nucleophilic reactivity of sulfur and oxygen atoms in enzymatic reactions catalyzed by the enzyme hydroxymethylase.[6]

We have reported the synthesis of $OHCH_2UdR$[7] and its ether derivatives.[8] 5-Methoxymethyl-2′-deoxyuridine has been found to have marked inhibitory effect against DNA viruses[9] and $OHCH_2UdR$ has shown potent cytotoxic activity against mouse tumor cells.[10] 5-Mercapto-2′-deoxyuridine has been shown to be effective in the treatment of skin neoplasms[11] and 5-mercaptomethyluracil has been reported to have marked inhibitory activity against transplantable tumors.[12] In this communication we report the synthesis and some biological properties of 5-mercaptomethyldeoxyuridine.

5-Mercaptomethyluracil (I) on treatment with 4 mol equiv of trimethylsilyl chloride in benzene and triethylamine as the acid acceptor gave tris(trimethylsilyl)-5-mercaptomethyluracil (II) as an oil in good yield (82%). The structure of II was confirmed by ir and NMR spectra. Coupling of II with 3,5-di-*O*-(*p*-toluoyl)-2-deoxy-D-*erythro*-pentofuranosyl chloride under fusion conditions at 185–195°[7,8,13] in the presence of a rapid stream of nitrogen yielded an anomeric mixture of blocked nucleosides (70–78%). After removal of the silyl groups by moist nitrogen in ethyl acetate, the β anomer of the protected nucleoside III was recovered as a crystalline solid (13–15%). The β configuration was assigned on the basis of a characteristic triplet for the anomeric proton at δ 6.33 with a coupling constant ($J_{1',2'} = J_{1',2''} = 6.95$ Hz).[14,15] Desulfurization of III using Raney nickel followed by deblocking resulted in almost complete conversion to β-thymidine (VI). This served to confirm the site of attachment of 2-deoxy-D-*erythro*-pentofuranosyl and also its configuration.[14] Attempts to separate the anomeric blocked nucleosides by chromatography of mother liquors on silica gel column or by preparative TLC[7,8] were not successful. After removal of blocking groups by sodium methoxide in methanol, deionization in the presence of a reducing atmosphere (using H_2S to prevent the formation of V), and chromatography on neutral alumina, IV was recovered as an amorphous powder in 70% yield. IV was stable when stored as a solid or in solution in the presence of thiol at room temperature. However, it was readily converted to its corresponding disulfide (V) upon dissolution and standing in distilled water or methanol. The structure and configuration of V was established from analysis and NMR spectrum. In compound V, the signal of the anomeric protons (H-1′) appeared as a triplet at δ 6.57 and exhibited a coupling constant ($J_{1',2'} = J_{1',2''} = 6.7$ Hz). The signals of all other protons in V appeared at the expected positions and the observed integration ratios were equal to the calculated values.



The relative antiviral potency of IV, V, iododeoxyuridine (IUdR), and cytosine arabinoside (ara-C) against the herpes virus of infectious bovine rhinotracheitis (IBR) was determined in secondary BFK cells using the assay procedure described earlier.[9] Reduced glutathione (GSH, 100 μg/ml) was incorporated in the medium in all experiments involving IV to maintain the integrity of the sulfhydryl group. The results of three identical experiments are summarized in Table I. When IV was added to the cell cultures 18 hr prior to virus infection, its activity against IBR virus was equal to that of IUdR and ara-C. When added simultaneously with virus infection, IV showed slightly less activity than IUdR and ara-C. The antiviral potency of IV was further decreased when it was added 18 hr postinfection, whereas both IUdR and ara-C retained significant activity at the higher concentration. At the higher concentration, the antiviral activity of ara-C was usually accompanied by some cytotoxicity. However, IV did not produce any observable cytotoxic effects at concentrations up to 500 μg/ml. Since IV was not capable of inhibiting viral growth after cell culture infection, these results suggest that it may have potential only as a prophylactic rather than a therapeutic antiviral agent. IV failed to show any activity against infectious canine hepatitis (ICH) virus in dog kidney (DK) cells whereas IUdR at 280 μg/ml gave a one log inhibition of viral cytopathic effect. V did not exhibit significant activity in any of the tests and GSH was devoid of antiviral activity.

The acute toxicity of IV appears to be quite low. Intraperitoneal administration of IV at 600 mg/kg in Swiss mice

did not produce any gross lesions or mortality up to 1 week after injection.

Experimental Section

Melting points were observed with a Gallenkamp apparatus and are uncorrected. Specific rotations were measured (1-dm tube) with a Perkin-Elmer Model 141 automatic digital read out polarimeter. The NMR spectra were recorded on a Varian Associates A-100 spectrometer using tetramethylsilane (Me_4Si) as a standard (unless otherwise indicated). All compounds were dissolved in dimethyl sulfoxide (Me_2SO-d_6). The ir spectra were measured with a Perkin-Elmer Model 421 spectrophotometer. A Unicam spectrophotometer, Model SP800, was used to measure the complete uv absorption spectra. Microanalyses were performed by Dr. F. M. Strauss, Microanalytical Laboratory, Oxford. Silica gel (HF_{254}) was purchased from E. Merck, Darmstadt, Germany. The TLC was carried out on silica gel plates (0.3 mm) at 22° and spots were detected under uv illumination. The R_f values are ±0.05. Evaporations under diminished pressure were performed on a rotary evaporator at 35°. All chemicals and solvents were of reagent grade. 5-Hydroxymethyluracil was synthesized by base-catalyzed hydroxymethylation of uracil.[16]

5-Mercaptomethyluracil (I). I was prepared by the method of Sorolla and Medrek.[12] After two crystallizations from aqueous methanol it was obtained as a microcrystalline powder; the NMR spectrum was at δ 11.11 (1 H, s, 1-NH), 11.48 (1 H, s, 3-NH), 7.81 (1 H, s, 6-H), 3.70 (2 H, s, 5-CH_2–), 2.98 ppm (1 H, s, proton of mercapto group 5-CH_2SH). The uv absorption spectrum and melting point were the same as reported.

2,3,5-Tris(trimethylsilyl)-5-mercaptomethyluracil (II). To a stirring suspension of I (4.74 g, 30 mmol) in anhydrous benzene (100 ml) was added trimethylchlorosilane (13.04 g, 120 mmol). After 15 min, triethylamine (12.4 g, 120 mmol) was added dropwise over a period of 30 min and the reaction mixture was stirred at room temperature for 20 hr. The precipitated triethylamine hydrochloride was collected by filtration and washed with benzene (3 × 20 ml). The filtrate and washings were combined and the solvent was evaporated in vacuo at 35° to yield the crude product as a viscous oil (11.2 g). The oil on distillation at 118° (0.1 mm) gave II (9.21 g, 82%): ir[17] ν max (liquid film) 1037 (SiOC), 830 and 1249 [$Si(CH_3)_3$], 1425 cm^{-1} (CH_2S–) and no absorption between 2550–2600 cm^{-1} (free SH). The NMR (solvent CCl_4; formic acid external to locate trimethylsilyl groups) was at δ 3.02 [9 H, s, 2- or 4-$Si(CH_3)_3$], 2.96 [9 H, s, 2- or 4-$Si(CH_3)_3$], 2.89 [9 H, s, 5-$SSi(CH_3)_3$], 6.09 ppm (2 H, s, 5-CH_2–); to locate the 6-position proton of the pyrimidine ring (Me_4Si lock) at δ 5.81 (1 H, s, 6-H) and 3.48 ppm (2 H, s, 5-CH_2–); integration ratio 1:2:27.

1-[2-Deoxy-3,5-di-*O*-(*p*-toluoyl)-β-D-*erythro*-pentofuranosyl]-5-mercaptomethyluracil (III). A mixture of 3,5-di-*O*-(*p*-toluoyl)-2-deoxy-D-*erythro*-pentofuranosyl chloride[18] (16.2 g, 42 mmol) and II (14.96 g, 40 mmol) was mixed and fused at 185–195° under a continuous stream of nitrogen.[8] The reaction mixture was cooled, and the gummy residue was dissolved with stirring in 100 ml of EtOAc. Nitrogen saturated with water was bubbled in EtOAc for 1 hr to decompose the silyl groups. The organic layer was washed with water (3 × 30 ml) to remove unreacted I, dried over anhydrous Na_2SO_4, and filtered through a bed of Celite–charcoal. The solvent was removed under reduced pressure and the mixture of nucleosides contaminated with small amounts of sugar by-products was obtained as a slightly yellowish fluffy powder (18.71 g). The residue was dissolved in CH_2Cl_2 (20 ml) and slowly added to 800 ml of *n*-hexane with vigorous stirring at 4°. The filtrate which contained mostly sugar by-products was discarded and the precipitated nucleosides were collected by filtration (16.0 g, 78.5%). Crystallization from EtOAc–cyclohexane yielded the crude product (3.45 g, 17%, mp 195–200°). Recrystallization from EtOAc–CH_2Cl_2 furnished the pure nucleoside III (2.22 g, 10%): mp 202–203°; $[\alpha]^{25}D$ 83.6° (*c* 0.11, $CHCl_3$); uv λ max (EtOH, 95%) 242 mμ (ε 32,000) and a broad shoulder at 265–270 nm; ir (KBr pellet) ν max 2574 cm^{-1} (SH); NMR at δ 11.52 (1 H, s, 3-NH), 7.65 (1 H, s, 6-H), 2.63 (1 H, t, proton of CH_2SH group; partially buried under the solvent), 4.6 (2 H, d, 5-CH_2– with ring), 6.33 (1 H, t, $J_{1',2'} = J_{1',2''}$ = 6.95 Hz, 1'-H), 5.63 (1 H, m, 3'-H overlapped by 5'-CH_2– group), 2.46–2.48 (2 H, m, solvent and 2'-, 2''-H), 7.3 and 7.39 (4 H, d, 2 sets, two phenyl rings 2-H and 6-H), 7.87 and 7.95 (4 H, d, 2 sets, two phenyl rings 3-H and 5-H), 2.40 ppm (6 H, two *p*-methyl groups of benzene ring); mass spectra parent peak (mass) 510. Anal. ($C_{26}H_{26}O_7N_2S$) C, H, N, S.

This product moved as a homogeneous spot on TLC (R_f 0.35) using solvent $CHCl_3$–cyclohexane–MeOH (45:45:10). Examination of the mother liquors by TLC showed that it contained only nucleosides and optical rotation indicated that it was a mixture of α and β anomers. However, attempts on the separation of blocked nucleoside anomers by crystallization, column chromatography using silica gel,[7] or by preparative TLC[8] were unsuccessful.

1-(2-Deoxy-β-D-*erythro*-pentofuranosyl-5-mercaptomethyluracil (IV). (i) By Hydrolysis of III. Compound III (510.5 mg, 1 mmol) was dissolved in MeOH (20 ml) and the solution was made anaerobic by bubbling nitrogen. NaOMe (350 mg) was added and the reaction mixture was refluxed for 90 min under a gentle stream of nitrogen. The solution was cooled and hydrogen sulfide was bubbled for 2 hr. Dowex 50 (H^+, X-8, 200–400 mesh) was added and the solution (pH 3–4) was stirred for 30 min. The resin was separated by filtration and washed with MeOH (3 × 10 ml). The filtrate and washings were combined, the solvent was removed under reduced pressure, the gummy mass was dissolved in deaerated water (10 ml), and precipitated toluic acid was collected by filtration. The filtrate was extracted with ether (3 × 20 ml) to remove remaining toluic acid and the aqueous layer was lyophilized to give a white fluffy powder (225.0 mg). A small quantity (10 mg) of the product was dissolved in MeOH (2.0 ml) containing β-mercaptoethanol (0.05%) and was examined by TLC chromatography using $CHCl_3$–MeOH (80:20) as developer. In addition to IV (R_f 0.65) there was an additional quenching spot (R_f 0.12) due to formation of its disulfide V (10–20%). Further purification of IV was accomplished by chromatography on a column of neutral alumina. The powder (2i5.0 mg) was dissolved in MeOH (5.0 ml) made previously anaerobic by bubbling nitrogen and the solution was layered on a column (1.5 × 20 cm) of neutral alumina equilibrated previously with oxygen-free MeOH. The column was eluted with deaerated MeOH (300 ml) under a slight pressure of nitrogen and the effluent was kept oxygen free by bubbling nitrogen slowly. The solvent was evaporated under reduced pressure; the gummy residue was dissolved in oxygen-free water (5 ml) and lyophilized to give a white fluffy powder (170 mg). The product moved essentially as a homogeneous spot on TLC (R_f 0.65) in solvent ($CHCl_3$–MeOH, 80:20) containing β-mercaptoethanol at a final concentration of 0.5%. V was insoluble in MeOH and was retained on the column.

(ii) By Reduction of V. To a solution of V (109 mg, 0.2 mmol) in MeOH (10 ml), dithiothreitol (50 mg) was added and the resulting mixture was left at 25° for 30 min. Chromatography of the reaction mixture on TLC using solvent $CHCl_3$–MeOH (80:20) showed a single quenching spot (R_f 0.65); the starting material in this solvent system has R_f 0.12; uv λ max (0.1 *M* phosphate buffer, pH 7.0) 274 nm (ε 7800); ir (KBr pellet) ν max 2568 cm^{-1} (SH). Anal. ($C_{10}H_{14}N_2O_5S$·H_2O) C, H, N. IV was readily oxidized to its corresponding dilsulfide (V) in solution, but no decomposition was observed when IV was stored as a powder in desiccator.

Bis[1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-5-mercaptomethyluracil] (V). IV (274.2 mg, 1 mmol) was dissolved in water (10 ml) and a gentle stream of oxygen was bubbled through aqueous solution for 20 hr. Chromatography (TLC) using $CHCl_3$–MeOH (80:20) for development showed the presence of a single slow-moving quenching spot (R_f 0.12). Water was removed under reduced pressure and the residue was crystallized from MeOH–EtOAc to give V (240 mg): mp 208–209°; uv λ max (0.1 *M*, phosphate buffer, pH 7.0) 273 nm (ε 14,100); NMR at δ 8.23 (1 H, s, 6-H), 6.57 (1 H, t, $J_{1',2'} = J_{1',2''}$ = 6.7 Hz, 1'-H), 5.62 (1 H, broad peak, hydroxyl group 3'-OH), 5.0–5.6 (1 H, m, OH or 5'-CH_2OH overlapped with 3'-OH), 4.66 (1 H, m, 3'-H), 4.19 (1 H, q, 4'-H), 3.98 (2 H, t, 5'-CH_2–), 2.87 (2 H, d, 5-CH_2–), 11.10–11.80 (1 H, broad peak, 3-NH) and peak at 2.63 ppm (1 H, t, 5-SH) was absent. Anal. ($C_{20}H_{26}N_4S_2O_{10}$) C, H, N, S. The compound moved as a homogenous spot on TLC (R_f 0.12) in solvent $CHCl_3$–MeOH (80:20). The disulfide bond was readily cleaved by dithiothreitol to give IV at room temperature (vide supra). However, V was not reduced by mercaptoethanol or glutathione up to a concentration of 1 m*M*.

1-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-5-methyluracil (β-Thymidine) (VI). III (51.1 mg, 0.1 mmol) was added to methanol (30 ml) containing Raney nickel (1.0 g) and refluxed for 4 hr. The catalyst was collected by filtration and washed with MeOH (2 × 10 ml). Washings were combined with filtrate, solvent was removed in vacuo, and blocking groups were removed using sodium methoxide (vide supra). β-Thymidine was recovered from the reaction mixture (yield, 85%). Specific rotation, uv absorption, and NMR spectra were identical with an authentic sample.

Biological Data. Cell Culture Studies. Antiviral activity

against IBR virus and ICH virus was determined using secondary BFK cells and a dog kidney (DK) cell line (38th passage) obtained from Connaught Laboratories, Toronto, Canada. The cells were grown in CulturStat (minimal essential medium MEM, Earle Base) containing 10% inactivated fetal calf serum. Maintenance medium contained the following components by volume: 10% MEM, 10% $NaHCO_3$ (4.4% stock solution), 4% inactivated fetal calf serum, 1% nonessential amino acids, 1% penicillin (100 U/ml), 1% streptomycin (100 μg/ml), and 73% double-distilled deionized water. The cells were treated with trypsin or EDTA.[18] Confluent monolayers of BFK or DK cells were grown in plastic disposable microplates having flat-bottomed cups (Micro-Test II) and were used for all antiviral chemotherapy experiments.[9] The cytotoxicity of each compound was determined microscopically.[9]

Stability of IV in the Presence of Reducing Agents. IV is readily oxidized to the corresponding bis compound V, which is essentially devoid of antiviral activity. Therefore, it was necessary to incorporate a reducing agent into the assay medium in cell culture studies. In order to find a reducing agent devoid of cytotoxicity and that would also prevent the oxidation of IV, the effects of four reducing agents on cell cultures were studied microscopically.[9] The stability of IV was followed by TLC chromatography. Dithiothreitol (DTT) and GSH prevented oxidation of IV at concentrations of 50 μg/ml or higher, whereas mercaptoethanol was only partially effective up to a concentration of 78 mg/ml and ascorbic acid was ineffective. However, DTT was toxic to cells above 50 μg/ml, whereas GSH showed no cytotoxicity up to 250 μg/ml. Therefore, on the basis of the above results, GSH was incorporated at 100 μg/ml into the medium for all cell culture studies.

Toxicity Studies. IV (10 mg/ml) was dissolved in phosphate buffer (0.15 *M*, pH 7.2) containing GSH (3 mg/ml) and administered intraperitoneally to 20–25-g Swiss mice. Controls were injected with an equal volume of the above buffer.

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Synthesis and Central Nervous System Effects of 8,9-Dihydro[1]benzazepino[3,2,1-*jk*][1,4]benzodiazepin-1(2*H*)-ones and [1]Benzazepino[3,2,1-*jk*][1,4]benzodiazepin-1(2*H*)-ones

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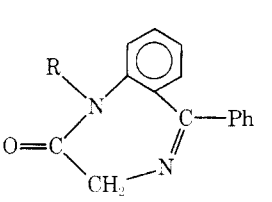
A series of 4-alkyl-8,9-dihydro[1]benzazepino[3,2,1-*jk*][1,4]benzodiazepin-1(2*H*)-ones and brominated derivatives was synthesized. Two approaches for the synthesis of 4-alkyl[1]benzazepino[3,2,1-*jk*][1,4]benzodiazepin-1(2*H*)-ones and brominated derivatives are described. All compounds were evaluated for CNS activity. None showed significant activity. The results obtained indicate that in the case of the 1,3-dihydro-5-phenyl-2*H*-1,4-benzodiazepin-2-one a phenyl group at the 1 position causes a fall in CNS activity not only when it is *free* but also when *fused* to the benzodiazepine system.

A large number of 1,4-benzodiazepines have been synthesized by a variety of methods,[1] and extensive data on their pharmacological activity[2] have been accumulated over the past 15 years. Interesting observations[3] were also made by investigating the 1,4-benzodiazepinones of type 1.

Although it was recognized that a substituent larger than methyl at the 1 position had a negative effect on central nervous system (CNS) activity, our work has been centered on the fusion of tricyclic rings to the seven-membered diazepine ring system thus resulting in novel 1,4-benzodiazepinones of type 2. These may be viewed as conformationally rigid analogs of CNS inactive compound 3 in which the precise spatial relationship between rings A and B can be varied by appropriate modification of the central ring. Of the several variants examined by Dreiding model studies, the most promising group for CNS biological activity appeared to be X = CH_2—CH_2, X = CH=CH, and X = S. In fact, these molecules could exhibit interesting CNS properties as they have stereochemistry and/or delocalization of electrons[4] different from the inactive compound 3. Furthermore, they contain a condensed three-ring system similar to several CNS active compounds (10,11-dihydrodibenz[*b*,*f*]azepine, dibenz[*b*,*f*]azepine, and phenothiazine).



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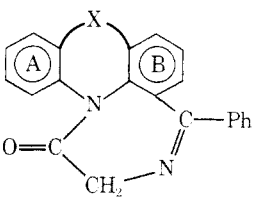

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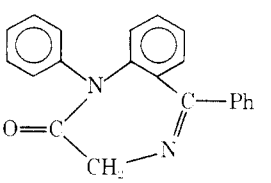

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