Synthesis and Biological Properties of 4-Amino- and 4-Bromo-4-norpyridoxol. New Approaches for the Modification of the 4 Position of Vitamin B_6

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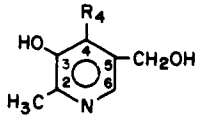
4-Amino-4-norpyridoxol, a new key intermediate for the modification of the 4 position of vitamin B_6, has been obtained by an unusual photochemical rearrangement of pyridoxal oxime. It has also been synthesized starting from 3,α^5-*O*-dibenzylpyridoxal, which was converted to 3,α^5-*O*-dibenzylpyridoxamide. The latter, on Hoffman reaction, gave the desired 3,5-blocked 4-amino derivative. Several derivatives of this analogue have been prepared, and its existence in the amino tautomeric form has been established by NMR spectroscopy. A modified Sandmeyer reaction on 4-amino-4-norpyridoxol gave the 4-bromo analogue, which was found to be moderately active as an inhibitor of mouse mammary adenocarcinoma cells grown in cell culture, whereas the 4-amino analogue was not active at 10^{-4} M. Other analogues containing electron-withdrawing and electron-donating substituents in the 4 position of pyridoxine were also tested.

The modification of the 4 position in vitamin B_6 has been fruitful for obtaining potent antagonists of this growth factor.[1,2] Thus, the replacement of the 4-formyl group of pyridoxal with an inert methyl group has resulted in the classical and most widely studied antagonist of vitamin B_6, namely 4-deoxypyridoxal (4-DOP, 1, R_4 = CH_3).[2] More recently, we have described the synthesis and antagonist properties of vitamin B_6 analogues containing



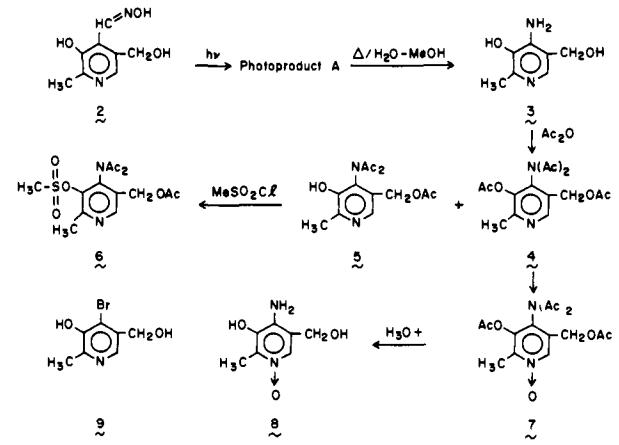
I

the more reactive vinyl (1, R_4 = CH=CH_2)[3,4] and ethynyl (1, R_4 = C≡CH) groups in this position. The introduction of the amino group into the 4 position (1, R_4 = NH_2) provides an example of an analogue with a substituent resembling, in size, the methyl group in 4-DOP (1, R_4 = CH_3) but with greater electron-donating properties. The analogue could also be considered to be the next lower homologue of pyridoxamine (1, R_4 = CH_2NH_2), one of the biologically active forms of vitamin B_6. Hence it is also a potential substrate of pyridoxal phosphokinase, which is an important consideration in the design of vitamin B_6 antagonists, since most of them have to be activated by in vivo phosphorylation in order to exert their antagonist activity.[1]

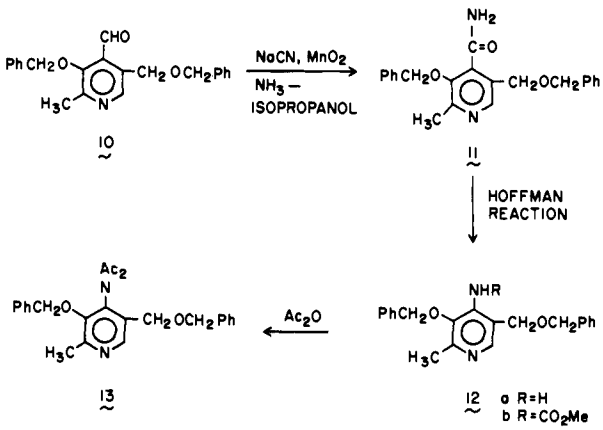
The target compound 3 was obtained by an unusual photochemical rearrangement of pyridoxal oxime, giving "photoproduct A". The latter on treatment with aqueous methanol gave 3, which was acetylated mainly to the tetraacetyl derivative 4, and some triacetyl derivative 5 (Scheme I). The total yield was 45%. The nature of the photoproduct, the mechanism of the photochemical reaction, and some of the by-products of the reaction have been discussed in a preliminary communication.[5] The triacetyl derivative 5 has been characterized by mesylation to give 6. The *N*-oxide of the target compound 8 has been obtained by oxidation of the tetraacetyl derivative 4 with *m*-chloroperbenzoic acid to 7 and subsequent deacetylation with acid. The *N*-oxide was prepared in the hope of improving the yield in the Sandmeyer reactions necessary for the synthesis of 4-halogen-substituted analogues. Starting with 3, the yield of the 4-bromo analogue was 27%. The yield could not be improved by using the *N*-oxide 8 as the starting material and subsequent reduction of the *N*-oxide with triphenylphosphine.

In order to prepare the 4-amino analogue 3 on a larger scale, a different procedure has been developed, starting

Scheme I



Scheme II



with 3,5-di-*O*-benzylpyridoxal (10, Scheme II). Advantage has been taken of the method developed by Gilman[6] for the conversion of aldehydes to amides using the oxidation of the initially formed cyanohydrin and subsequent reaction with ammonia. This reaction has been carried out on 10 in isopropyl alcohol without isolation of the intermediate and gave the blocked amide 11 in an excellent yield. The Hoffman reaction on 11 has been performed under various reaction conditions. In absolute methanol[7] a high yield of the urethane 12b has been obtained, which on saponification gave a quantitative yield of the amine 12a. In order to obtain the amine directly, the Hoffman reaction in nonhydroxylic solvents has been carried out. In aqueous THF or dioxane, the yield of the amine was poor. Under these conditions the reaction gives rise to

appreciable quantities of the blocked 4-pyridoxic acid along with the starting material. If, however, the reaction is carried out in DMF, the amine **12a** is obtained in a satisfactory yield. The blocked amine was characterized by preparation of the diacetyl derivative **13**. Hydrolysis of **13** with hot 2 N HCl gave the target compound **3** in an excellent yield. Many earlier attempts to prepare **3** by the application of the Hoffman reaction to pyridoxic acid amide in which the hydroxyl groups were not blocked were not successful.

4-Aminopyridine and other related heterocyclic amines are known to exist as amino–imino tautomers, as shown by NMR spectroscopy[8] and other evidence.[9] The tautomerism of the aminopyridine **12a** was investigated by NMR spectroscopy in solvents in which amino–imino forms have been detected. This compound **12a** was found to exist in the amino form only, as evidenced by the presence of a singlet due to the NH_2 protons at δ 4.68 in $CDCl_3$ and at δ 5.50 in Me_2SO-d_6, respectively. Formation of the *N*-diacetyl derivative **13** is also consistent with the presence of the amino form as the sole tautomer. The same applies to the deblocked compound **3**.

The synthesis of the 4-amino analogue of vitamin B_6 by the two routes should facilitate the introduction of various other substituents into this position in addition to bromine.

Biological Data. The two analogues **3** and **9** were tested as inhibitors of the growth of mouse mammary adenocarcinoma (TA-3) cells grown in cell culture in Eagle's medium containing a minimal amount of pyridoxal (1×10^{-7} M) to sustain growth.[10] The 4-amino analogue **3** did not inhibit growth of TA-3 cells at 10^{-4} M, whereas the 4-bromo analogue was inhibitory with an ID_{50} of 1×10^{-5} M. The 5-bromomethyl analogue of **3** (obtained by heating **4** with aqueous HBr) at 10^{-4} M concentration inhibited growth to approximately 30%. It was of interest to compare the effect of other small electron-withdrawing and -donating groups on the inhibitory activity of vitamin B_6 analogues modified in the 4 position. The most active analogue, 4-trifluoromethyl-4-norpyridoxol [3-hydroxy-2-methyl-4-trifluoromethylpyridine-5-methanol (**1**), R_4 = CF_3],[12] representing a very similar group both in size and electron-withdrawing effect to the 4-bromo derivative **9**, also had a similar ID_{50} of 2×10^{-5} M. The oxime of pyridoxal (**2**) was also found to inhibit at 8×10^{-5} M. The 4-hydroxy analogue of pyridoxal, 3,4-dihydroxy-2-methylpyridine-5-methanol (**1**, R_4 = OH),[13] representing another analogue with an electron-donating group, was found to be devoid of activity against TA-3 cells at a concentration of 10^{-4} M. Formation of *N*-oxides also decreased the inhibitory activity. Thus the *N*-oxide of 4-DOP, which was prepared by N-oxidation of the acetylated 4-DOP and subsequent deacetylation, had an ID_{50} of 5×10^{-5} M, whereas **8** was not active at 10^{-4}. It should be pointed out that both the introduction of electron-withdrawing substituents and N-oxidation reduce the ability of the ring nitrogen to become protonated. Nevertheless, the major reason for the decrease in the inhibitory activity of analogues may be related to their ability to be phosphorylated in vivo and thus become inhibitors of pyridoxine-P oxidase and possibly competitors for the pyridoxal phosphate cofactor site of the many enzymes that are dependent on this cofactor. Although the 4-modified analogues have the potential to be phosphorylated at the 5 position, this property has to be determined by experiment.

Experimental Section

TLC (silica gel) was used routinely as described earlier.[14] Ir spectra were determined with a Perkin-Elmer 457 spectrophotometer and NMR spectra with a Varian A-60A instrument, using 8–15% solutions in $CDCl_3$, Me_2SO, or D_2O; positions of peaks are expressed in hertz from Me_4Si, or from dioxane (222 Hz), as an internal standard. Peaks are assigned on the basis of previous work.[15]

Irradiation of Pyridoxal Oxime. A solution of pyridoxal oxime (1.00 g) in methanol (95 ml, absolute) was irradiated in an atmosphere of N_2 with a Hanovia 8A-1 quartz lamp for 16 h. The original spectrum (λ_{max}^{MeOH} 218, 260, 325 nm) changed to λ_{max}^{MeOH} 228, 314 nm. The solution was concentrated in vacuo, giving an oily product, TLC of which (1:1 $CHCl_3$–MeOH) showed the presence of at least four compounds with R_f's of 0.55, 0.65, 0.70, and 0.85. In order to isolate the major product (R_f 0.85), the residue was washed with ether and ethyl acetate and then was extracted with hot ethanol. The solid thus obtained (mp 228–230°) gave essentially one spot on TLC. During attempted purification, the produce was converted to a stable product, **3**, of R_f 0.4 (λ_{max}^{MeOH} 228, 314 nm), which was isolated as described here. For preparative purposes this conversion to **3** has been carried out by heating the irradiation product with aqueous methanol (1:1, 60 ml) on a steam bath for 5 h, followed by evaporation under vaccuum.

Isolation of 3-Acetoxy-5-(acetoxymethyl)-4-(diacetylamino)-2-methylpyridine (4). The crude reaction mixture consisting mainly of **3** was heated for about 30 min on a steam bath with pyridine (50 ml) and acetic anhydride (40 ml) and kept at room temperature overnight. After evaporation in vacuo, the residue was taken up in water (40 ml) and extracted with chloroform (5 × 50 ml). The combined chloroform extracts were dried ($MgSO_4$) and filtered. After removal of the solvent in vacuo, the solid residue was crystallized from $CHCl_3$–Et_2O, giving 9.80 g (45%) of the tetraacetyl derivative **4**, mp 126–127°. Anal. ($C_{15}H_{18}N_2O_6$) C, H, N.

4-Amino-3-hydroxy-5-(hydroxymethyl)-2-methylpyridine (3) Hydrochloride. Compound **4** (200 mg) was heated on a steam bath with 5 N HCl (30 ml) for 20 h, the water was evaporated in vacuo, and the residue was crystallized from ethanol: mp 109° (92%); NMR (D_2O) 144 (CH_3), 470 (C_6-H). The methylene group was obscured by the HDO peak: mass spectrum *m/e* 154 nm (M^+). Anal. ($C_7H_{11}ClN_2O_2$) C, H.

From the mother liquors in the preceding hydrolysis reaction, a small amount of 4-pyridoxic acid lactone was isolated and was recrystallized from methanol. It was identified by its ir, NMR, and mass spectra, as well as its behavior on TLC.

5-(Acetoxymethyl)-4-(diacetylamino)-3-methanesulfoxy-2-methylpyridine (6). Mother liquors from the preparation of **4** were chromatographed on a silica gel column. Elution with chloroform–ether gave 62 mg of **4** and 55 mg of 5-(acetoxymethyl)-4-(diacetylamino)-3-hydroxy-2-methylpyridine (**5**, R_f 0.3), which was crystallized from ether–chloroform: mp 156–157°; mass spectrum *m/e* 280 nm (M^+). The compound was allowed to react with methanesulfonyl chloride in pyridine, giving the 3-*O*-methanesulfonyl derivative **6**: mp 93° (from methanol–ether); NMR ($CDCl_3$) 125 (2-CH_3), 304 (5-CH_2OH), 523 (C_6-H), 141 [$N(COCH_3)_2$], 163 (5-$COCH_3$), 300 (SO_2CH_3). Anal. ($C_{14}H_{18}N_2O_7S \cdot 0.5H_2O$) C, H.

3-Acetoxy-5-(acetoxymethyl)-4-(diacetylamino)-2-methylpyridine *N*-Oxide (7). 3-Acetoxy-5-(acetoxymethyl)-4-(diacetylamino)-2-methylpyridine (**4**, 96.6 mg), in a chloroform solution (45 ml), was allowed to react with *m*-chloroperoxybenzoic acid (44 mg) overnight at room temperature. The chloroform solution was shaken with 10% aqueous $NaHCO_3$, followed by 10% $NaHCO_3$ solution and water, and was then dried. Evaporation and crystallization gave 101 mg of the *N*-oxide: mp 105°; NMR ($CDCl_3$) 132 (2-CH_3), 147 (acetate CH_3's), 303 (5-CH_2OH), 515 (C_6-H). Anal. ($C_{15}H_{18}N_2O_7$) C, H, N.

4-Amino-3-hydroxy-5-(hydroxymethyl)-2-methylpyridine *N*-Oxide (8) Hydrochloride. Compound **7** (34 mg) in 10 ml of 6 N HCl was stirred at room temperature for 6 h. Solvent was removed in vacuo by repeated evaporation with water. The residue consisted of two products (R_f 0.40 and 0.38 in 1:1 $CHCl_3$–MeOH). After several crystallizations from methanol–ether, the lower R_f compound **8** was obtained as a crystalline product: mp 145° (softens at 130°); NMR (D_2O) 147 (2-CH_3), 485 (C_6H); CH_2OH was obscured by the HDO peak. Anal. ($C_7H_{11}ClN_2O_3$) C, H, N.

4-Bromo-3-hydroxy-5-(hydroxymethyl)-2-methylpyridine (9) Hydrobromide. A solution of 3 hydrochloride (190.5 mg, 1.00 mmol) in 48% aqueous HBr (14 ml) was cooled to −10°, and solid $NaNO_2$ (180 mg, 2.6 mmol) was added during 15 min of stirring. After stirring for 30 min at room temperature, the solvent was evaporated in vacuo. This was followed by repeated evaporations from water. The residue was sublimed at 5×10^{-5} Torr (Hg diffusion pump) at an oil-bath temperature of 165–171°. Recrystallization from a methanol–ether mixture yielded 80 mg (27%) of the bromo analogue: mp 245° dec; NMR (D_2O) 157 (2-CH_3), 287 (5-CH_2OH), 491 (C_6-H). Anal. ($C_7H_9Br_2NO_2$) C, H, Br, N.

4-Amino-5-(bromomethyl)-3-hydroxy-2-methylpyridine Hydrochloride. Compound 4 (102 mg) was heated in 48% aqueous HBr (5 ml) for 5 min and subsequently stirred for 48 h at room temperature. The solvent was flash-evaporated, and the residue was recrystallized from ethanol, giving 83 mg of the product, mp 220° dec. Anal. ($C_7H_{10}Br_2N_2O_2$) C, H, N.

3,α^5-O-Dibenzyl-4-pyridoxamide (11). To 10 ml of isopropyl alcohol saturated with ammonia, powdered NaCN (245 mg, 5 mmol) was added at 0°. After 5 min, 3,α^5-O-dibenzylpyridoxal[16] (10, 347 mg, 1 mmol) was added, followed by MnO_2 B[17] (1.7 g, 20 mmol), which was added in two equal portions 10 min apart. After 4 h at 0°, the solution was filtered, using Celite filter aid, and the solvent was evaporated. The amide was extracted with ethyl acetate, and the extract was washed with water and dried over anhydrous $MgSO_4$. It was filtered and was evaporated to a white solid. The amide was crystallized from acetone (mp 140–142°, 320 mg, 88%): NMR ($CDCl_3$) 150 (2-CH_3), 276, 279 ($2CH_2$), 395 ($CONH_2$, broad, slow exchange with D_2O), 444, 446 ($2C_6H_5$), 506 (6-H); ir ν_{max}^{KBr} 3380, 3165 (two NH peaks), 1648 (amide I band), 1680 cm^{-1} (amide II band); uv $\lambda_{max}^{95\%\ alcohol}$ 278 nm (ϵ 5070). Anal. ($C_{22}H_{22}N_2O_3$) C, H, N.

Hoffman Reaction on the Amide 11. NaOH (300 mg) was taken up in water (3.2 ml), the solution was cooled in an ice–salt bath, and bromine (0.13 ml) was added dropwise. When the temperature of the bath became 0°, 0.32 ml of NaOBr solution was transferred into a small flask cooled in ice. The amide 11 (100 mg, 0.28 mmol), dissolved in MeOH (0.6 ml), was added to this flask, and the mixture was stirred well for 15 min at 0°. The flask was now transferred to a water bath at 70–80°; stirring was continued for 1 h. After cooling, the reaction mixture was extracted with ethyl acetate, washed with water, dried over anhydrous $MgSO_4$, filtered, and evaporated to dryness. TLC indicated the formation of two compounds. They were separated by column chromatography, using a silica gel column and eluting with ethyl acetate. The higher *Rf* compound was found to be the urethane 12 (R = CO_2CH_3, mp 104–105°, 72 mg, 66.2%), the lower R_f compound being the amine 12 (R = H, 25 mg, 26.3%).

Urethane 12 (R = CO_2CH_3): NMR ($CDCl_3$) 151 (2-CH_3), 222 (OCH_3), 272, 274 ($2CH_2$), 293 (CH_2), 432 (NH) (exchanged with D_2O), 445 ($2C_6H_5$), 503 (6-H); ir ν_{max}^{KBr} 3260 (NH), 1705 (C=O), 1260 cm^{-1} (COC); uv $\lambda_{max}^{95\%\ alcohol}$ 267 nm (ϵ 3924). Anal. ($C_{23}H_{24}N_2O_4$) C, H, N.

The 4-amino compound 12, R = H (20 mg, 0.06 mmol), was taken up in anhydrous ether (2 ml), and ethereal HCl was added to convert the free base to its hydrochloride. Excess HCl was removed by evaporation under vacuum with anhydrous ether. The hydrochloride was crystallized from alcohol–ether (mp 115–116°, 18 mg, 81%): NMR ($CDCl_3$) 144 (2-CH_3), 276, 278 ($2CH_2$), 294 (CH_2), 299 (4-NH_2) (exchanged with D_2O), 441, 443 ($2C_6H_5$), 478 (6-H); ir ν_{max}^{KBr} 3280, 3170 (NH), 1641 (NH_2 deformation); uv $\lambda_{max}^{95\%\ alcohol}$ 277 nm (ϵ 1.04×10^4); $\lambda_{max}^{pH\ 7}$ 272 nm; MS molecular ion peak agreed with molecular weight of the compound. Anal. ($C_{21}H_{23}ClN_2O_2$) C, H, N.

3,α^5-O-Dibenzyl-4-(diacetylamino)-4-norpyridoxol [3-(Benzyloxy)-5-(benzyloxymethyl)-4-(diacetylamino)-2-methylpyridine, 13]. The amine 12a (25 mg, 0.75 mmol) was taken up in pyridine (1 ml), acetic anhydride (1 ml) was added, and the mixture was warmed for a short period. It was left at room temperature for 4 h. The pyridine was completely removed, and the diacetyl derivative 13 was extracted with ethyl acetate. The ethyl acetate layer was washed with water, dried (Na_2SO_4), filtered, and evaporated to an oil (24 mg, 83%): NMR ($CDCl_3$) 132 [$2CH_3C(=O)$–], 154 ($2CH_3$), 271 282, 291 ($3CH_2$), 444, 445 ($2C_6H_5$), 512 (6-H); ir ν_{max}^{neat} 1725, 1700 (C=O), 1225 cm^{-1} (COC).

The oily material (20 mg, 0.048 mmol) was taken up in anhydrous ether (1 ml), and ethereal HCl was added to convert the free base into its hydrochloride. After removing excess HCl, the compound was crystallized from acetone (mp 119–120°, 17 mg, yield 78%): NMR ($CDCl_3$) 133 [$2CH_3C(=O)$–], 163 ($2CH_3$), 265, 276, 296 ($3CH_2$), 441 ($2C_6H_5$), 514 (6-H); ir ν_{max}^{KBr} 1737, 1709 (C=O), 1215 cm^{-1} (COC); uv $\lambda_{max}^{95\%\ alcohol}$ 280 nm (ϵ 5.01×10^3). Anal. ($C_{25}H_{24}N_2O_4Cl$) C, H, Cl.

3,α^5-O-Dibenzyl-4-amino-4-norpyridoxol [4-Amino-3-(benzyloxy)-5-(benzyloxymethyl)-2-methylpyridine, 12a]. To NaOBr (1 ml) at 0° a solution of the amide 11 (25 mg) in DMF (0.2 ml) was added, and the reaction mixture was stirred at 0° for 5 min, after which it was heated at 70–80° in a water bath for 90 min. After cooling, the amine was extracted with ethyl acetate; the ethyl acetate solution was washed with water, dried ($MgSO_4$), and evaporated, yielding 17 mg of the amine (74%). In the ir spectrum and TLC, the amine was identical with the authentic sample of **12a** obtained by the Hoffman reaction in aqueous methanol as already described.

3,α^5-O-Dibenzyl-4-(methoxycarbonylamino)-4-norpyridoxal [3-(Benzyloxy)-5-(benzyloxymethyl)-4-(methoxycarbonylamino)-2-methylpyridine, 12b]. The amide 11 (72 mg, 0.2 mmol) was added to a solution of Na (10 mg) in absolute methanol (0.8 ml) kept at 0°. The mixture was stirred and kept below 0° while bromine (40 mg, 0.25 mmol) was added dropwise. When all of the amide had dissolved, the solution was refluxed for 1 h. The alcohol was evaporated under vacuum, and the residue was taken up in water and neutralized with acid. The methylurethane **12b** was extracted with ethyl acetate, and the organic layer was washed with water, dried ($MgSO_4$), evaporated to a solid, and crystallized from ethanol–ether (71 mg, yield 92%). It was identical with the sample obtained by the Hoffman reaction in aqueous methanol (see above). The methylurethane **12b** (60 mg, 0.2 mmol) was taken up in 10% NaOH solution (8 ml) and heated to 80–90% for 4 h. The reaction mixture was cooled and neutralized, and the amine was extracted with ethyl acetate. The extract was washed with water, dried over anhydrous $MgSO_4$, filtered, and evaporated to an oil, **12a** (50 mg, yield 98%). Ir and NMR spectra compared exactly with those of the authentic sample.

4-Amino-4-norpyridoxol [4-Amino-3-hydroxy-2-methylpyridine-5-methanol, 3] Hydrochloride. (a) The amino compound **12a** (30 mg, 0.09 mmol) was taken up in 2 N HCl (5 ml), and the reaction mixture was heated on a steam bath for 16 h. Excess HCl and benzyl alcohol were removed by evaporation in vacuo, and 4-amino-4-norpyridoxol (3) hydrochloride was crystallized from a mixture of alcohol and ethyl acetate (mp 320–321° dec, 17 mg, yield 88%). The ir spectrum is indistinguishable from that of the authentic sample prepared via the photochemical reaction: NMR (Me_2SO-d_6) 150 (2-CH_3), 274 (5-CH_2), 445 (4-NH_2) (broad, exchanged with D_2O), 474 (6-H, OH's) (very broad underneath 5-CH_2 around 270); uv $\lambda_{max}^{0.1\ N\ NaOH}$ 298 nm (ϵ 6.19×10^3); $\lambda_{max}^{0.1\ N\ HCl}$ 276 nm (ϵ 1.1×10^4); $\lambda_{max}^{pH\ 7}$ 283 nm (ϵ 6×10^3), 308 (8.10×10^3). (b) The methylurethane **12b** (20 mg, 0.051 mmol) was taken up in 3 N HCl (5 ml), and the reaction mixture was heated on a steam bath for about 20 h. Excess HCl and benzyl alcohol were removed under vacuum, and the 4-amino-4-norpyridoxol hydrochloride (8 mg, yield 83%) was crystallized from alcohol–ether.

Acknowledgment. This study was supported in part by research grants (CA-08793, CA-13038, and PO1-CA-16056) from the National Institutes of Health. We are indebted to Dr. M. T. Hakala and Miss A. I. Mulhern of this department for the biological evaluation of the compounds. We wish to thank Dr. John A. Montgomery for sending us a sample of compound 1, R_4 = CF_3.

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Cysteine Derivatives with Reactive Groups as Potential Antitumor Agents

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Cysteine derivatives having diazo ketone and chloro ketone functions have been prepared. In order to effect adequate protection for modifying the carboxyl group, cysteine was converted to a thiazolidine derivative I, which was then converted to the *N*-acetyl derivative VII. The active ester method or activation with DCC yielded the diazo ketone derivative VIII. Similar treatment of the parent compound I with DCC led to a self-condensation reaction giving a diketopiperazine VI. The diazo ketone derivative VIII has been used in preparing α-chloro ketone derivative X and a homologue of cysteine. Deblocking N-acetylated thiazolidine derivatives with various reagents did not proceed satisfactorily. Interaction of the N-acetylated blocked ester XII with trifluoroacetic acid opened the thiazolidine ring to give the N-acetylated blocked diester XIII and other products. The chloro ketone derivative X was found to have a moderate inhibitory activity against mouse mammary adenocarcinoma in cell culture. *S*-(1-Adamantyl)-L-cysteine was prepared and was found to be inactive.

Diazo ketone or diazo ester analogues of amino acids include well-known antibiotics, such as DON (6-diazo-5-oxo-L-norleucine) and azaserine, which have been found to possess antitumor activity. Azaserine was shown to inhibit specifically a glutamine transferase involved in purine biosynthesis and was considered by Baker to be the first example of a classical-type antimetabolite causing specific active-site-directed irreversible inhibition.[1] One of the effects that have been shown for DON is inhibition of the biosynthesis of D-glucosamine by interfering with formation of the latter compound from fructose 6-phosphate. In this step, glutamine is the amino-group donor, and thus DON is considered to be a glutamine antagonist.[2] Analogues of other amino acids having similar groups may have interesting biological properties, particularly with respect to their possible antiproliferative action. For instance, it has been found that certain cancer cells have an absolute requirement for cysteine.[3] A derivative of cysteine, *S*-trityl-L-cysteine, was found to have antileukemic activity.[4,5] These findings gave impetus for our studies of cysteine analogues, although other nonessential amino acids, such as L-serine, were also found to be required for growth of neoplastic cells.[6-9]

The main purpose of this study was to synthetize diazo ketone derivatives of cysteine. We also considered them as potential intermediates for the synthesis of homologues and halo ketones related to this amino acid.

We considered both the blocked and the deblocked compounds of potential biological interest, particularly since some highly substituted cysteine derivatives have been shown to be active,[4,5] and since partial or complete deblocking could be accomplished in vivo. Aldehydes are known to block the vicinal SH and NH_2 functions of cysteine, and being readily prepared by condensing cysteine with appropriate aldehydes to form 2-substituted thiazolidine-4-carboxylic acids, they appeared attractive for this purpose. Thus 2-phenylthiazolidine-4-carboxylic acid, 2-(*p*-nitrophenyl)thiazolidine-4-carboxylic acid, 2-(*p*-anisyl)thiazolidine-4-carboxylic acid, and 2-(*p*-tolyl)-thiazolidine-4-carboxylic acid were synthesized from L-cysteine by using the appropriate aromatic aldehydes. Initial study indicated that these compounds can be cleaved by methanolic hydrazine solution, giving substituted phenylhydrazones and cystine. The formation of cystine can be avoided by adding β-mercaptoethanol to the reaction mixture, and cysteine is recovered.

On the basis of its stability and the results of cleavage studies, we selected 2-(*p*-tolyl)-(*R*)-thiazolidine-4-carboxylic acid (I, Scheme I) for further modification. Initially, we attempted to prepare the diazo ketone II from I via the acid chloride. Nevertheless, even using a mild method (DMF and $SOCl_2$),[10] the acid chloride synthesis was not successful. King and his co-workers[11] have also been unsuccessful in making the acid chloride from thiazolidine-4-carboxylic acid by the conventional method. Next the mixed anhydride procedure was tried with ethyl chloroformate and diazomethane.[12] This resulted in preferential N-acylation, giving III. When the diazomethane was omitted from the reaction mixture, *N*-(ethoxycarbonyl)-2-(*p*-tolyl)-(*R*)-thiazolidine-4-carboxylic acid (IV) was obtained. Treatment of I with ethereal diazomethane gave the expected methyl ester V. Application of the dicyclohexylcarbodiimide method for the synthesis of diazo ketones[13] yielded the biomolecular cyclic amide VI as the only identifiable product. Treatment of I with DCC alone also gave VI along with unidentified products.

In order to avoid complications, the NH group in I had to be blocked by acetylation of I to VII (Scheme II). The configuration of C-2 in this key intermediate has been