6-Aminopyrazolo[4,3-*c*]pyridin-4(5*H*)-one, a Novel Analogue of Guanine

Kenneth W. Ehler, Roland K. Robins, and Rich B. Meyer, Jr.*[1]

ICN Pharmaceuticals, Inc., Nucleic Acid Research Institute, Irvine, California 92715. *Received February 11, 1976*

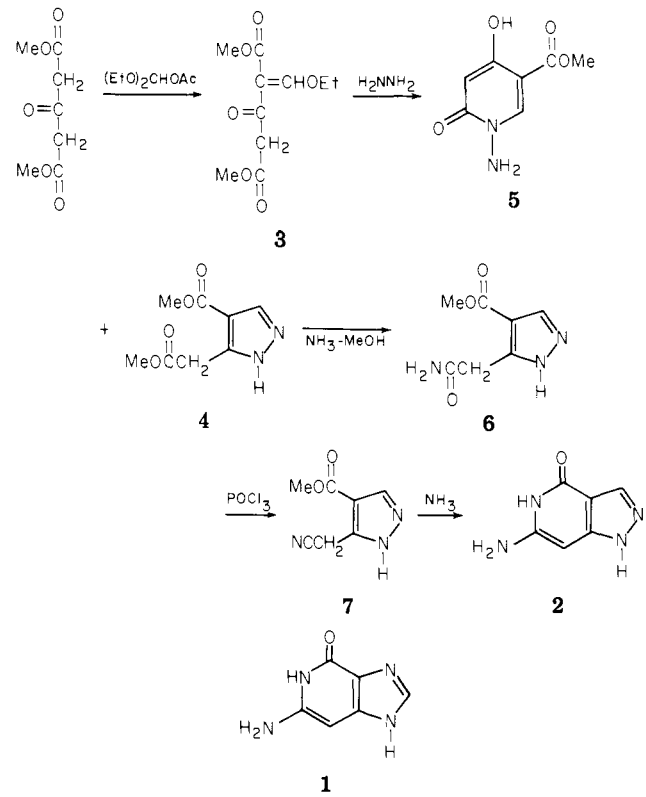
Treatment of dimethyl α-ethoxymethylidineacetonedicarboxylate with hydrazine gave methyl 3-(methoxycarbonylmethyl)pyrazole-4-carboxylate which, upon ammonolysis and dehydration, afforded methyl 3-(cyanomethyl)pyrazole-4-carboxylate. This compound, when heated with liquid ammonia, gave 6-aminopyrazolo[4,3-*c*]pyridin-4(5*H*)-one, a new guanine analogue, which did not possess any of the potent antiviral activity shown by 6-aminoimidazo[4,5-*c*]pyridin-4(5*H*)-one (3-deazaguanine).

The recent report of the potent antiviral and antitumor activity of 3-deazaguanine[2] [1, 6-aminoimidazo[4,5-*c*]-pyridin-4(5*H*)-one] has prompted the investigation of other 3-deazaguanine analogues. Thus the synthesis of 6-aminopyrazolo[4,3-*c*]pyridin-4(5*H*)-one (**2**) was undertaken (Scheme I).

Condensation of hydrazine with dimethyl α-ethoxymethylidineacetonedicarboxylate (**3**) gave the required starting material for the synthetic procedure, methyl 3-(methoxycarbonylmethyl)pyrazole-4-carboxylate (**4**), albeit in only 6% yield. The major product of this reaction (36%) was found to be the product of cyclization to the ester function, methyl 1-amino-4-hydroxypyridin-2-one-5-carboxylate (**5**). This is similar to the ring closure Errera reported[3] for the reaction of dimethyl α-ethoxymethylidineacetonedicarboxylate with ammonia. The subsequent steps in the procedure were similar to the preparation of 3-deazaguanine.[2] Treatment of **4** with methanolic ammonia gave 78% of methyl 3-(carbamoylmethyl)pyrazole-4-carboxylate (**6**), in analogy with room temperature ammonolysis of similar diesters.[2,4] Dehydration of the amide with phosphoryl chloride gave 90% of methyl 3-(cyanomethyl)pyrazole-4-carboxylate (**7**). Ammonolysis of the ester and cyclization to the desired product was affected by heating **7** with liquid ammonia in a bomb. This compound was fluorescent, as was 3-deazaguanine.[2]

When tested[5] for its ability to inhibit the cytopathic effect of herpes virus type 1, parainfluenza virus type 3, and rhinovirus type 13 on KB cells in culture, it was found that compound **2** and its precursors had no activity. This surprising result was in marked contrast to the pronounced activity of **1**, which was highly effective against all of these viruses.[2a]

Scheme I



Experimental Section

NMR spectra were recorded on a Hitachi Perkin-Elmer R-20A spectrometer using Me_4Si as an internal standard. Elemental Analyses were performed by Galbraith Labs., Knoxville, Tenn. Thin-layer and column chromatography were performed using Woelm silica gel. The spots on TLC were visualized using a *tert*-butyl hypochlorite spray followed by KI–starch (Unispray aerosol reagent, ICN Pharmaceuticals, Inc., Life Sciences Group, Cleveland, Ohio). IR spectra in KBr disks were run on a Perkin-Elmer 257 and UV spectra were recorded on a Cary 15.

Methyl 3-(Methoxycarbonylmethyl)pyrazole-4-carboxylate (4). Dimethyl acetonedicarboxylate (24.9 g, 0.145 mol) and diethoxymethyl acetate (23.3 g, 0.145 mol) were refluxed in a 100 °C oil bath for 2.5 h and then evaporated in vacuo using a water aspirator. Addition of 100 ml of benzene followed by evaporation was repeated three times. The residual oil was dissolved in 25 ml of MeOH and cooled to 0–5 °C. With continued cooling, 2.6 ml (75 mmol) of 97+% hydrazine in 50 ml of MeOH was added with cooling. The solution, after standing at ambient temperature overnight, was filtered and evaporated to dryness, and the residue was dissolved in a minimum of 2:1 benzene–ether. Column chromatography of this solution on a 4 × 36 cm column of silica gel packed in and eluted with the same solvent gave **4** as the first major product off the column. Evaporation of the solvent and crystallization of the residue from EtOAc gave 1.8 g (6.2%) of product: mp 103–105 °C; R_f 0.56 (ether development); ν_{max} 3250, 1735, 1720 cm^{-1}; NMR ($CDCl_3$) 3.67 δ (s, 3-H), 3.76 (s, 3-H), 3.93 (s, 2-H), 8.19 (s, 1-H), 13.4 (br s, 1-H). Anal. ($C_8H_{10}N_2O_4$) C, H, N.

The solid residue from filtration of the methanol solution after addition of the hydrazine was recrystallized from MeOH to give 9.6 g (36%) of **methyl 1-amino-4-hydroxypyridin-2-one-5-carboxylate (5)**: mp 159–160 °C; NMR (Me_2SO-d_6) δ 3.84 (s, 3-H), 5.81 (s, 1-H), 6.12 (br s, 2-H), 8.38 (s, 1-H), 10.83 (br s, 1-H); λ_{max}^{pH1} 228 nm (ε 27 600), 257 (10 200); λ_{max}^{pH7} 232 nm (ε 46 400); λ_{max}^{pH11} 233 nm (ε 54 000); ν_{max} 1700, 1665 cm^{-1}. Anal. ($C_7H_8N_2O_4$) C, H, N.

Methyl 3-(Carbamoylmethyl)pyrazole-4-carboxylate (6). A solution of 1.0 g (5.0 mmol) of **4** in 5 ml of MeOH saturated with NH_3 was allowed to stand 2 days and then evaporated. The residue was triturated with ether to give 0.72 g (78%) of product: mp 163–164 °C; ν_{max} 3350, 3160, 1710, 1680 cm^{-1}; NMR (Me_2SO-d_6) δ 3.78 (s, 5-H, $-CH_3$ and $-CH_2-$), 7.20 (d, 2-H, NH_2), 8.03 (s, 1-H), 13.3 (s, 1-H). Anal. ($C_7H_9N_3O_3$) C, H, N.

Methyl 3-(Cyanomethyl)pyrazole-4-carboxylate (7). A mixture of 1.5 g of **6** (8 mmol) in 30 ml of $POCl_3$ was refluxed 1 h and then evaporated to dryness. Toluene (20 ml) was added and evaporated three times. With dry ice bath cooling, concentrated NH_4OH was added until the pH of the solution was maintained at 6.5. The precipitated crystalline product was collected on a filter and recrystallized from EtOAc to give 1.2 g (90%) of **7**: mp 145 °C; ν_{max} 3160, 2160, 1715 cm^{-1}; NMR (Me_2SO-d_6) δ 3.74 (s, 3-H), 4.20 (s, 2-H), 8.41 (s, 1-H), 13.5 (br

s, 1-H). Anal. ($C_7H_7N_3O_2$) C, H, N.

6-Aminopyrazolo[4,3-*c*]pyridin-4(5*H*)-one (2). A mixture of 20 ml of anhydrous NH_3 and 550 mg (3.3 mmol) of **7** was heated 90 h in a steel bomb at 110 °C. Evaporation of the NH_3 gave a residue which was extracted with several portions of hot MeOH. The combined extracts were evaporated to give 50 mg (9%) of pure **2**: gradual decomposition over 250 °C; λ_{max}^{pH1} 253 nm (ϵ 7100); $\lambda_{max}^{pH7and11}$ 272 nm (ϵ 13000); R_f (cellulose TLC developed in 5% aqueous NH_4HCO_3) 0.33; NMR (Me_2SO-d_6) δ 3.73 (br s, 1-H, HOD), 5.41 (s, 1-H), 5.78 (s, 2-H), 7.82 (s, 1-H), 10.5 (br s, 1-H). Anal. ($C_6H_6N_4O \cdot 0.5H_2O$) C, N; H: calcd, 4.43; found, 4.98.

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References and Notes

(1) Address correspondence to this author at the Department of Pharmaceutical Chemistry, School of Pharmacy, University of California, San Francisco, Calif. 94143.
(2) (a) P. D. Cook, R. J. Rousseau, A. M. Mian, R. B. Meyer, Jr., P. Dea, G. Ivanovics, D. G. Streeter, J. T. Witkowski, M. G. Stout, L. N. Simon, R. W. Sidwell, and R. K. Robins, *J. Am. Chem. Soc.*, **97**, 2916 (1975); (b) P. D. Cook, R. J. Rousseau, A. M. Mian, P. Dea, R. B. Meyer, Jr., and R. K. Robins, *ibid.*, **98**, 1492 (1976).
(3) G. Errera, *Chem. Ber.*, **31**, 1682 (1898).
(4) R. K. Robins, J. H. Horner, C. V. Greco, C. W. Noell, and C. G. Beames, Jr., *J. Org. Chem.*, **28**, 3041 (1963).
(5) Antiviral tests were performed according to the procedure described by R. W. Sidwell and J. H. Huffman, *Appl. Microbiol.*, **22**, 797 (1971).

Estrogen Potentiating Activity of Two Spiro Compounds Having Approximately Similar Molecular Dimensions to Stilbestrol

Neil S. Doggett,* David J. Bailey, and Tariq Qazi

Welsh School of Pharmacy, University of Wales Institute of Science and Technology, Cardiff, Great Britain.
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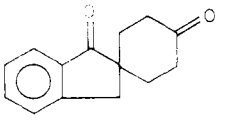
Pharmacological investigation of members of a series of synthetic spiro derivatives with similar molecular dimensions to stilbestrol revealed that two compounds, spiro[cyclohexane-1,2′-tetralin]-1,4′-dione and spiro[cyclohexane-1,2′-indan]-1,4′-diol, exhibited a marked ability to potentiate stilbestrol at doses which had no intrinsic estrogenic activity. It is postulated that such compounds may be of use in reducing the side effects associated with estrogen therapy.

It has previously been reported that one member (**1**) of a series of estrogenically inactive spiro derivatives with similar molecular dimensions to pharmacologically active estrogens potentiated the effects of stilbestrol on the immature mouse uterus.[1] This paper describes two further compounds, spiro[cyclohexane-1,2′-tetralin]-1,4′-dione (**3a**) and spiro[cyclohexane-1,2′-indan]-1,4′-diol (**4**), which were produced as a result of extending the series. They are of particular interest in view of a marked increase in potency compared with the compound originally described.

Chemistry. 2,2-Bis(β-ethoxycarbonylethyl)tetralone (**2a**) was prepared by a Michael condensation between ethyl acrylate and α-tetralone. Ring closure was then accomplished by means of a single-step modification of the Dieckmann reaction to give spiro[cyclohexane-1,2′-tetralin]-1,4′-dione (**3a**).

A similar sequence of reactions starting with α-indanone afforded spiro[cyclohexane-1,2′-indan]-1,4′-dione (**3b**) which was then reduced using sodium borohydride to give spiro[cyclohexane-1,2′-indan]-1,4′-diol (**4**).



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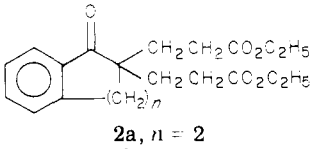

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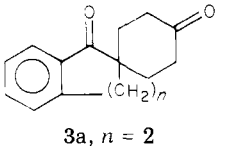
b, n = 1



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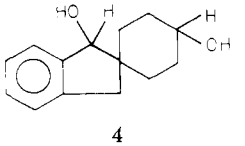

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Pharmacological Activity. In order to quantify estrogenic activity, a bioassay procedure based on the method first described by Rubin et al.[2] was employed. This method utilizes the increase in uterine weight produced by both synthetic and naturally occurring compounds possessing estrogenic activity in immature mice. It is both sensitive and precise and yields easily quantifiable data from simple objective measurements. Tests were carried out to detect any estrogenic activity of the spiro derivatives under investigation and also to estimate their ability to potentiate or antagonize the effects of a standard estrogen (stilbestrol).

Compound **4** and to a lesser extent **3a** produced a small although significant ($p < 0.001$) increase in the uterine ratio compared with arachis oil controls. This effect was, however, minimal when compared with that produced by a very much smaller dose (0.01 mg/kg) of stilbestrol. Furthermore, the response tended to fall off following a 100-fold increase in the dose and this may indicate an inhibitory effect at higher concentrations (Table I).

In contrast to the very small intrinsic estrogenic activity, both derivatives exhibited a marked ability to potentiate stilbestrol. Furthermore, this effect was produced at dose levels which alone had no significant estrogenic effect (Table II). Compound **3a** was the more potent in this respect and extending the dose range to encompass much smaller doses revealed that as little as 1 μg/kg produced a twofold increase in the stilbestrol response. This effect was even more marked at 10 μg/kg but began to diminish at doses in excess of this.

The adverse reactions associated with estrogen therapy are well documented and include thromboembolism, stroke, myocardial infarction, hepatic tumors, gall bladder disease, hypertension, and endometrical cancer. The type of compound described here is of interest since it may allow smaller doses of estrogens to be used clinically with a consequent reduction in the incidence and/or severity of these side effects.

At the present time the mechanism by which the spiro derivatives bring about their estrogen potentiating effects