TABLE II
INHIBITORY EFFECTS OF β-AMINO KETONES ON PYRUVIC ACID OXIDATION

No.	Inhibition of pyruvic[a] acid, 5×10^{-4} M	
	−NAD	+NAD
I	46.1 ± 0.47	32.3 ± 0.63
II	44.3 ± 0.41	45.3 ± 0.76
III	76.4 ± 0.47	49.1 ± 1.40
IV	34.5 ± 0.70	21.8 ± 1.00
V	61.8 ± 0.94	22.6 ± 0.47
VI	10.3 ± 1.10	13.4 ± 0.65

[a] Values indicate mean per cent inhibition calculated from the decrease in oxygen uptake per 100 mg of fresh weight per hour. All values are mean of four duplicate runs.

pound studies, β-hexamethyleneimono-4-piperidinopropiophenone (III) was found to be the most potent inhibitor of pyruvic acid (Table I).

The inhibition produced by the β-amino ketones was reduced in the presence of added NAD except in the case of II and VI. In these instances, the addition of NAD in no way altered their inhibitory effects. These results are in agreement with those of other investigators in which similar protection was observed by added NAD.[13] It was interesting to note that the inhibition produced by the β-amino ketones having a dimethyl substitutent (VI) could not be protected by added NAD. At present, it is difficult, and, indeed with these limited data, impossible to define the exact role that the substituents play in permitting the added NAD to afford protection against the inhibitory effects of the β-amino ketones. Further studies in progress dealing with the effect of β-amino ketones of this type on other dehydrogenases using purified enzyme preparations may possibly elucidate the site and mechanism of action of these β-amino ketones.

Experimental Section[14]

β-Amino Ketones.—To a mixture of 4-piperidinoacetophenone hydrochloride (0.05 mole), the appropriate secondary amine hydrochloride (0.05 mole), and paraformaldehyde (1.5 g) were added 10 ml of EtOH and two drops of concentrated HCl. The reaction mixture was refluxed on a water bath for 2 hr. At the end of this period 1 g of paraformaldehyde was added and the mixture was further refluxed for 6 hr. After distilling off excess EtOH under vacuum, 50 ml of dry MeAc was added to the viscous mass thus obtained. The product obtained after overnight refrigeration was removed by filtration and recrystallized from EtOH–MeAc. The purity of these compounds was ascertained by their melting points, elemental analysis, and infrared spectra. Data for the various amino ketones are recorded in Table I.

Acknowledgments.—The authors wish to express their thanks to Professor K. P. Bhargava, Head of the Department of Pharmacology and Therapeutics, for providing necessary facilities and to Mr. Nafasat Husain Rahmani for technical assistance. Grateful acknowledgment is made to the Council of Scientific and Industrial Research, New Delhi, for financial assistance.

(13) P. K. Seth and S. S. Parmar, *Can. J. Physiol. Pharmacol.*, **43**, 1019 (1965).

(14) Melting points were taken in capillary tubes on a Thomas–Hoover apparatus and are uncorrected. Elemental analyses were obtained from Galbraith Laboratories, Inc., Knoxville, Tenn. Infrared spectra were recorded in Nujol mull on a Perkin-Elmer Model 137 Infracord spectrophotometer and were as expected.

Synthesis of 6-Trifluoromethyl-1,4-dihydrazinophthalazine

BRUNO CAVALLERI, ELVIO BELLASIO, AND ANNA SARDI

Research Laboratories, Lepetit S.p.A., Milan, Italy

Received August 11, 1969

In a previous paper[1] we described the synthesis of various 1-hydrazinophthalazines carrying a fluorine atom on different positions of the benzene ring, in order to investigate the effect of this substitution on the pharmacological activity. We have now prepared 6-trifluoromethyl-1,4-dihydrazinophthalazine dihydrochloride (V).

The preparation of V was accomplished according to the general procedure for the synthesis of hydrazinophthalazines[2,3] with minor modifications. 4-Trifluoromethylphthalic anhydride (I), described as a mobile liquid by Loev[4] and as a dark solid by Lombardino,[5] was obtained in a pure state by distillation *in vacuo*; a solid product resulted, mp 65°. By condensing I with hydrazine hydrate 6-trifluoromethyl-2,3-dihydro-1,4-phthalazinedione (II) was obtained. This was converted into 5-trifluoromethyl-1,4-dichlorophthalazine (III), which with an equivalent amount of sodium methoxide furnished 6-trifluoromethyl-1(4)-chloro-4(1)-methoxyphthalazine (IV). By action of hydrazine hydrate on IV the desired 6-trifluoromethyl-1,4-dihydrazinophthalazine was obtained and then transformed into the more stable dihydrochloride (V).

Pharmacology.—Compound V was studied for hypotensive activity in conscious renal hypertensive rats[6] and dogs[7] and in anesthetized normotensive dogs, for adrenergic blocking activity in dogs, and for cardiac action on isolated guinea pig hearts (Langendorff). Acute oral toxicity was investigated in mice and dogs.

Blood pressure was measured in renal hypertensive rats and dogs by an indirect method, before treatment by the oral route and 2 and 4 hr later, for 4 consecutive days; the minimal effective dose is 3 mg/kg in both species. A drop of 13 mm was produced even by 0.5 mg/kg iv. The hypotensive effect increases proportionally with dosage. In the anesthetized dogs a reduction of the pressor response to epinephrine was induced by doses as low as 0.1 mg/kg iv. In isolated guinea pig hearts doses of 5 and 20 μg increased the coronary flow by 21 and 50%, respectively, and did not modify the heart rate and amplitude of contractions.

The approximate LD_{50} by the oral route in mice is 280 mg/kg; in dogs it produced convulsions and death in three out of four animals treated with 100 mg/kg orally, while only sedation and anorexia were observed in two animals treated with 75 and 50 mg/kg.

Dihydrazinophthalazine was tested in comparison with V in hypertensive rats and dogs. In rats the minimal effective oral dose was 3 mg/kg. In two dogs oral dosing with 2 mg/kg induced a drop in blood

(1) B. Cavalleri and E. Bellasio, *Farmaco, Ed. Sci.*, **24**, 833 (1969).
(2) S. Biniecki and J. Z. Izdebski, *Acta Polon. Pharm.*, **15**, 421 (1957).
(3) A. Hirsch and D. Orphanos, *Can. J. Chem.*, **43**, 2708 (1965).
(4) B. Loev, *ibid.*, **42**, 1488 (1964).
(5) J. G. Lombardino, *J. Org. Chem.*, **32**, 1989 (1967).
(6) A. Grollmann, *Proc. Soc. Exp. Biol. Med.*, **57**, 102 (1944).
(7) H. Goldblatt, J. Lynch, R. F. Hanzel, and W. W. Summerville, *J. Exp. Med.*, **59**, 227 (1937).

pressure of about 20 mm the first day of treatment, but tachyphylaxis occurred on subsequent days. The oral LD_{50} in mice, calculated by the method of Litchfield and Wilcoxon,[8] was 380 mg/kg. Although preliminary, these data seem to lead to conclusion that trifluoromethyl substitution does not increase the hypotensive activity, whereas it induces a certain enhancement of toxicity.

Experimental Section[9]

4-Trifluoromethylphthalic Anhydride (I).—A solution of 7.9 g of 4-trifluoromethylphthalic acid[4,5,10] in 40 ml of Ac_2O was refluxed for 2 hr. Excess solvent was removed and the residue was distilled at 80–85° (0.4 mm) to give 7 g (95%) of I which solidified on standing, mp 62–65°. The purity of the product was checked by glpc (F and M Model 5750 apparatus equipped with a flame ionization detector and Moseley recorder Model 7172A; F and M stainless steel column 1.8 m × 2 mm i.d., filled with silicone rubber UCW 98 on Diatoport S 80–100 mesh (10:100), column temperature 120°, injector temperature 160°, detector temperature 160°, N_2 flow 35 cc/min), retention time 2 min 40 sec; ir, 1880 and 1790 (C=O), 1255 (C—O), 1330 and 1180 (CF_3), 885 cm^{-1} (CH arom).

6-Trifluoromethyl-2,3-dihydro-1,4-phthalazinedione (II).—A solution of 3.22 g (0.064 mole) of 98% hydrazine hydrate in 64 ml of AcOH was added slowly with cooling to a solution of 13.9 g (0.064 mole) of I in 540 ml of AcOH. The mixture was refluxed for 2 hr. After cooling the precipitate was filtered, washed with Et_2O, and crystallized from MeOH to afford 12.6 g (85%) of II: mp 298–300° dec; ir, 3300–2100 (N—H and O—H), 1670 (C=O), 1590 (C=N), 1315 and 1140 (CF_3), 870 and 820 cm^{-1} (CH arom); nmr (DMSO-d_6), τ 1.8–1.5 (multiplet 3 H, H arom), −1.6 to −2.1 (broad singlet, 2 H, OH and NH). *Anal.* ($C_9H_5F_3N_2O_2$) C, H, F, N; equiv wt: calcd, 230.14; found, 225 (pK_{MCS} = 6.8).[11]

6-Trifluoromethyl-1,4-dichlorophthalazine (III).—An intimate mixture of II (6.05 g, 0.026 mole) and 30 g of PCl_5 was placed in a glass liner of a bomb tube and heated at 170–175° (oil bath temperature) for 6 hr. After cooling the reaction mixture was added to 400 g of ice–water and neutralized with concentrated NH_4OH. The solid was filtered, washed with H_2O, dried *in vacuo*, and crystallized from $(i\text{-Pr})_2O$ to give 5.9 g (84%) of III, mp 129–130°. The product can be sublimed at 70° (0.2 mm). Ir spectra were as expected. *Anal.* ($C_9H_3Cl_2F_3N_2$) C, H, Cl, F, N.

6-Trifluoromethyl-1(4)-chloro-4(1)-methoxyphthalazine (IV).—A solution of 14.5 g (0.054 mole) of III in 290 ml of MeOH was added with stirring to a solution of 1.29 g (0.056 g-atom) of Na in 100 ml of MeOH at room temperature. After boiling at reflux for 45 min the solvent was evaporated *in vacuo* to dryness and the residue was extracted with $(i\text{-Pr})_2O$. The extract was filtered to remove insoluble material, treated with charcoal, and concentrated to crystallization, yield 11.3 g (79%), mp 133°. An analytical specimen was recrystallized from $(i\text{-Pr})_2O$, mp 135°. Ir and nmr spectra were as expected. *Anal.* ($C_{10}H_6ClF_3N_2O$) C, H, Cl, F, N.

6-Trifluoromethyl-1,4-dihydrazinophthalazine Dihydrochloride (V).—To 15 ml of 98% hydrazine hydrate and 15 ml of absolute EtOH heated at 40°, 23 g (0.088 mole) of IV was added, and the mixture was boiled at reflux for 2 hr. When the temperature rose to 85° solution occurred. After cooling, a red precipitate was filtered, washed with a little absolute EtOH, and dried *in vacuo* at 50° yielding 8.9 g (39%), mp 152–156°. Recrystallization of a portion of this material from absolute EtOH furnished an analytical sample of the base, mp 155–156°, ir and nmr spectra as expected. *Anal.* ($C_9H_9F_3N_6$) C, H, N; F: calcd, 22.09; found, 22.76.

The dihydrochloride (V) was prepared by adding Et_2O–HCl to a solution of the base in the minimum amount of warm absolute EtOH. The precipitate was filtered and recrystallized from 95% EtOH–Et_2O, yield 5.94 g (54%), mp 212° dec, tlc (on silica gel G buffered at pH 2.2 with McIlvaine reagent, developed with EtOH–H_2O 65:35, and visualized by spraying with an aqueous solution of 0.1 *N* J and then with concentrated H_2SO_4, or with an ammoniacal $AgNO_3$ solution) R_f 0.55, ir and nmr spectra as expected. *Anal.* ($C_9H_9F_3N_6 \cdot 2HCl$) C, H, F, N, Cl^-.

Acknowledgment.—The authors are indebted to Dr. A. Cometti for glpc analyses and to Dr. E. Baldoli and Dr. M. V. De Zulian for supplying some of the pharmacological data.

(8) J. T. Lichfield, Jr., and F. Wilcoxon, *J. Pharmacol. Exp. Therap.*, **96**, 99 (1949).

(9) Where analyses are indicated only by symbols of the elements, analytical results for those elements were within ±0.4% of the theoretical values. Melting points were determined in capillary tubes and are uncorrected. Ir spectra were determined with a Perkin-Elmer Model 137 spectrophotometer as Nujol mulls. Nmr spectra were recorded at 60 Mcps by a Varian A-60 spectrometer using TMS as internal standard (10.00 ppm) in the solvents indicated. Spectra not mentioned specifically were as expected. F analyses were performed as described by B. Cavalleri, E. Bellasio, and E. Testa, *Gazz. Chim. Ital.*, **96**, 227 (1966).

(10) When prepared according to ref 5, crude 4-trifluoromethylphthalic acid was used directly.

(11) Potentiometric titration in MCS–H_2O (4:1) solution with 0.1 *N* NaOH.

The Synthesis of the α and β Anomers of 1-(2-Deoxy-D-ribofuranosyl)-2-pyridone[1]

MATHIAS P. MERTES

The Department of Medicinal Chemistry, School of Pharmacy, The University of Kansas, Lawrence, Kansas 66044

Received June 23, 1969

One phase in the design of nucleoside analogs as potential anticancer agents has been directed to the preparation of deaza and deoxy models of essential metabolites.[2] The design of such models for the inhibition of thymidylate synthetase[3] prompted this study. This note describes the synthesis of 1-(2-deoxy-β-D-ribofuranosyl)-2-pyridone (**5**, 3-deaza-4-deoxy-2′-deoxyuridine). The ribofuranosyl analog of **5** has been prepared from the HgCl salt of 2-pyridone and by application of the Hilbert–Johnson reaction[4] of 2-benzoyloxypyridine with the protected ribofuranosyl chloride.[5] A recent report from Wagner and coworkers describes the synthesis of the title compounds from the Ag salt of 2-pyridone.[5b,c] Similarly, 3-deazauridine has been synthesized by Robins and Currie by the silyl method.[2b]

The synthesis of the intermediate **3** was accomplished in low yield by application of the Hilbert–Johnson reaction of 2-benzoyloxypyridine (**1b**) with 3,5-di(O-*p*-toluyl)-2-deoxy-D-ribofuranosyl chloride (**2**).[6] A higher yield (6%) of the α and β anomeric mixture (**3a** and **b**) was achieved by use of the HgCl salt **1a**. After chromatographic separation of the anomers the protected α anomer (**3a**) crystallized. Transesterification of **3a** followed by silica chromatography gave crystalline 1-(2-deoxy-α-D-ribofuranosyl)-2-pyridone (**4**).

(1) This research was supported by Research Grant CA-5639 and Career Development Award 1K3-CA-10,739 of the National Cancer Institute, National Institutes of Health.

(2) (a) For references see M. Mertes, J. Zielinski, and C. Pillar, *J. Med. Chem.*, **10**, 320 (1967); (b) M. J. Robins and B. L. Currie, *Chem. Commun.*, 1547 (1968).

(3) (a) M. Friedkin, *Ann. Rev. Biochem.*, **32**, 185 (1963); (b) R. L. Blakely, B. V. Ramasastri, and B. M. McDougall, *J. Biol. Chem.*, **238**, 3075 (1963); (c) P. Reyes and C. Heidelberger, *Mol. Pharmacol.*, **1**, 14 (1965); (d) M. J. S. Lomax and G. R. Greenberg, *J. Biol. Chem.*, **242**, 1302 (1967); (e) E. J. Pastore and M. Friedkin, *ibid.*, **237**, 3802 (1962).

(4) J. Pliml and M. Prystas in *Advan. Heterocyclic Chem.*, **8**, 115 (1967).

(5) (a) H. Pischel and G. Wagner, *Arch. Pharm.*, **300**, 602 (1967); (b) D. Heller and G. Wagner, *Z. Chem.* **8**, 415 (1968); (c) P. Nuhn, A. Zschunke, D. Heller, and G. Wagner, *Tetrahedron*, **25**, 2139 (1969).