[Contribution from the Warner-Chilcott Research Laboratories]

Some Pyridazonyl Substituted Aliphatic Ketones and Alcohols

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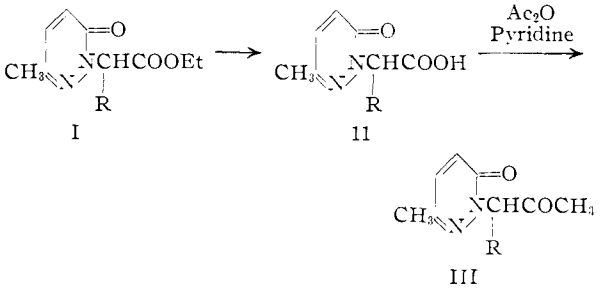
A series of 2-alkanones carrying a 6-pyridazonyl-1 substituent in either the 1- or 3-position has been prepared by the reaction of aliphatic acids, having the pyridazonyl group attached at the α-position, with acid anhydrides and pyridine. 3-Methyl-6-pyridazone has been shown to add to methyl vinyl ketone to give 4-(3-methyl-6-pyridazonyl-1)-butanone-2. 3-(3-Methyl-6-pyridazonyl-1)-butanone-2 has been converted to a secondary and a tertiary alcohol by reduction with aluminum isopropoxide and by a Grignard reaction, respectively.

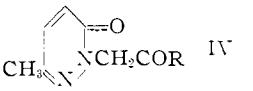
In a former publication[1] we have shown that 6-pyridazonyl-1-acetic acids readily undergo a decarboxylative acylation reaction with acetic anhydride and pyridine to form the corresponding methyl ketones. Since we had on hand, as a result of other work,[2] the esters of a number of new pyridazonyl substituted aliphatic acids, we decided to subject these acids to the decarboxylative acylation reaction to see whether the resultant ketones had any pronounced pharmacological activity. The description of the preparation of a series of such ketones and two related alcohols constitutes the subject matter of this communication.

Esters of the general formula I, where R is ethyl, *n*-propyl, *n*-butyl and *n*-amyl, have been



described in an earlier publication.[2] The ester where R is isopropyl has similarly been prepared. These esters have been hydrolyzed to give the acids, II, which have been treated with acetic anhydride and pyridine under reflux to give the ketones, III. In addition ethyl 3-phenyl-6-pyridazonyl-1-acetate[2] has been hydrolyzed to the corresponding acetic acid, which in turn was subjected to the decarboxylative acylation reaction with acetic anhydride and pyridine to give 1-(3-phenyl-6-pyridazonyl-1)-propanone-2. It was noted that this last reaction was not nearly as "cleancut" as the corresponding reaction in which the 3-position of the pyridazone nucleus carries a methyl group; the pure ketone was isolated only after tedious crystallization.

Since it is known that anhydrides other than acetic anhydride may be used in the decarboxylative acylation reaction,[3] we decided to try to prepare several ketones of the general structure IV. Accordingly we have treated 3-methyl-6-pyridazonyl-1-acetic acid with pyridine and propionic anhydride, *n*-butyric anhydride, isobutyric anhydride, *n*-valeric anhydride, isovaleric anhydride, methylethylacetic anhydride, *n*-hexanoic anhydride, *n*-heptanoic anhydride and *n*-octanoic anhydride and in each instance isolated the desired ketone. Pivalic anhydride failed to give an isolable amount of ketone. It is interesting that these ketones are all crystalline solids, whereas the ketones of type III are all viscous oils. It was also observed that the reaction using these longer chain anhydrides required a longer time than when acetic anhydride was used.



As an example of a possible alternative synthesis of ketones of type III, we carried out the alkylation of 3-methyl-6-pyridazone with 3-chlorobutanone-2. A moderate yield of ketone was obtained which was not, however, homogeneous; the refractive index was low (1.5063 compared with 1.5226 for 3-(3-methyl-6-pyridazonyl-1)-butanone-2),[1] and the semicarbazone required repeated crystallization before pure 3-(3-methyl-6-pyridazonyl-1)-butanone-2 semicarbazone was obtained. Since it seemed possible that the alkylation might proceed by an alternative route, *viz.*, preliminary dehydrohalogenation followed by addition of the pyridazone to the resultant methyl vinyl ketone to give 4-(3-methyl-6-pyridazonyl-1)-butanone-2, we have allowed 3-methyl-6-pyridazone to add to methyl vinyl ketone in ethanol in the presence of a trace of sodium ethoxide; 4-(3-methyl-6-pyridazonyl-1)-butanone-2 was obtained in good yield as a crystalline solid. When this material was used to seed the chlorobutanone alkylation product, no crystallization was induced; consequently, it is believed that the alkylation product does not contain a high proportion of the 4-substituted isomer.

In order to ascertain whether change of the functional group in the aliphatic part of these molecules from a ketone to secondary or a tertiary alcohol would influence any possible pharmacological action, we have prepared 3-(3-methyl-6-pyridazonyl-1)-butanol-2 (V) and 3-(3-methyl-6-pyridazonyl-1)-2-methylbutanol-2 (VI). Treatment of 3-(3-methyl-6-pyridazonyl-1)-butanone-2 (VII) with aluminum isopropoxide in isopropyl alcohol gave an 82% yield of crystalline 3-(3-methyl-6-pyridazonyl-1)-butanol-2.

The reaction of VII with methylmagnesium iodide gave crystalline VI in moderate yield, although the reaction did not go to completion and considerable starting ketone had to be separated from the tertiary alcohol chemically.

(1) J. A. King and F. H. McMillan, This Journal, **74**, 3222 (1952).

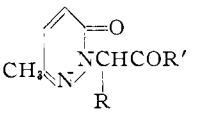
(2) F. H. McMillan, K. Kun, C. B. McMillan and J. A. King, *ibid.*, **78**, 407 (1956).

(3) F. E. Lehmann, A. Bretscher, H. Kuhne, E. Sorkin, M. Erne and H. Erlenmeyer, *Helv. Chim. Acta*, **33**, 1217 (1950).

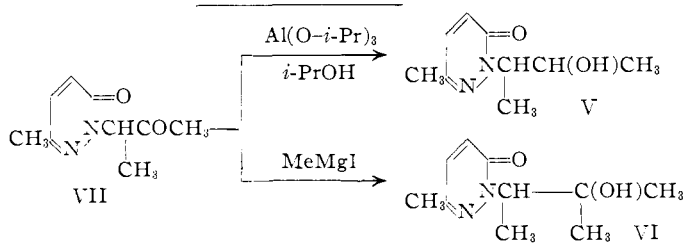
TABLE I

Acid	M.p., °C.	Empirical formula	Analyses, % Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
α-(3-Methyl-6-pyridazonyl-1)-*n*-butyric acid	191–191.5	$C_9H_{12}N_2O_3$	55.09	6.17	14.28	55.18	6.15	14.20
α-(3-Methyl-6-pyridazonyl-1-)-*n*-valeric acid	162–163	$C_{10}H_{14}N_2O_3$	57.13	6.71	13.33	57.25	6.65	13.22
α-(3-Methyl-6-pyridazonyl-1)-isovaleric acid	148–150	$C_{10}H_{14}N_2O_3$	57.13	6.71	13.33	57.21	6.65	13.08
α-(3-Methyl-6-pyridazonyl-1)-*n*-caproic acid	152–153	$C_{11}H_{16}N_2O_3$	58.91	7.19	12.49	58.99	7.29	12.55
α-(3-Methyl-6-pyridazonyl-1)-*n*-heptanoic acid	153–154.5	$C_{12}H_{18}N_2O_3$	60.48	7.61	11.76	60.41	7.53	11.81
3-Phenyl-6-pyridazonyl-1-acetic acid	227–228	$C_{12}H_{10}N_2O_3$	62.60	4.38	12.17	62.78	4.24	12.05

TABLE II



R	R′	Empirical formula	Yield, %	M.p., °C.	B.p. °C. (mm.)	Analyses, % Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
C_2H_5	CH_3	$C_{10}H_{14}N_2O_2$	62		84(0.05)	61.83	7.26	14.43	61.95	7.39	14.52
n-C_3H_7	CH_3	$C_{11}H_{16}N_2O_2$	58		96(0.10)	63.44	7.75	13.45	63.58	7.83	13.33
i-C_3H_7	CH_3	$C_{11}H_{16}N_2O_2$	56		83.5(0.02)	63.44	7.75	13.45	63.56	7.94	13.70
n-C_4H_9	CH_3	$C_{12}H_{18}N_2O_2$	51		118(0.35)	64.84	8.16	12.61	64.75	8.28	12.77
n-C_5H_{11}	CH_3	$C_{13}H_{20}N_2O_2$	60		135(0.60)	66.07	8.53	11.86	65.92	8.63	11.74
H	C_2H_5	$C_9H_{12}N_2O_2$	44	95–97		59.98	6.71	15.55	60.21	6.58	15.56
H	*n*-C_3H_7	$C_{10}H_{14}N_2O_2$	23	73–75		61.83	7.26	14.43	61.74	7.12	14.40
H	*i*-C_3H_7	$C_{10}H_{14}N_2O_2$	8	126–127		61.83	7.26	14.43	62.04	6.86	14.10
H	*n*-C_4H_9	$C_{11}H_{16}N_2O_2$	20	84.5–86		63.44	7.75	13.45	63.56	7.66	13.36
H	*i*-C_4H_9	$C_{11}H_{16}N_2O_2$	19	102–103		63.44	7.75	13.45	63.58	7.51	13.90
H	*s*-C_4H_9	$C_{11}H_{16}N_2O_2$	3	92.5–93.5		63.44	7.75	13.45	63.39	7.49	13.35
H	*n*-C_5H_{11}	$C_{12}H_{18}N_2O_2$	14	103–104		64.84	8.16	12.61	64.73	8.22	12.55
H	*n*-C_6H_{13}	$C_{13}H_{20}N_2O_2$	19	92–95		66.07	8.53	11.86	66.08	8.49	11.93
H	*n*-C_7H_{15}	$C_{14}H_{22}N_2O_2$	17	85–86		67.16	8.86	11.19	67.05	8.97	11.27



Experimental[4,5]

Preparation of Esters.—All of the pyridazonyl substituted esters have been previously reported[1,2] except ethyl α-(3-methyl-6-pyridazonyl-1)-isovalerate, which was prepared in 41% yield by the alkylation of 3-methyl-6-pyridazone with ethyl α-bromoisovalerate as previously described.[1,2] It boiled at 95–99° at 0.01 mm.

Anal. Calcd. for $C_{12}H_{18}N_2O_3$: C, 60.48; H, 7.61; N, 11.76. Found: C, 60.62; H, 7.78; N, 12.05.

Preparation of Acids.—The pyridazonyl substituted acids were prepared by heating the corresponding ethyl esters with an excess of 10% aqueous sodium hydroxide at reflux for 1 hr. The aqueous solution was cooled and acidified with concentrated hydrochloric acid. Each of the acids crystallized from the acidified solution in practically quantitative yield. Small samples were recrystallized from either water or very dilute ethanol for analysis. These acids are listed in Table I together with their melting points and analyses.

Preparation of Ketones.—In general the ketones were prepared by heating a mixture of the requisite acid, an anhydride and pyridine at reflux until carbon dioxide evolution ceased, after which the reaction was worked up in a suitable manner. A few representative examples are described in detail below; most of the ketones are described in Table II. The anhydrides used are all known; those which were not available commercially were prepared by fractionation of a mixture of the acid and acetic anhydride.

(4) Boiling points and melting points are uncorrected.

(5) Microanalyses were carried out by Miss Lorelinde Einstein.

3-(3-Methyl-6-pyridazonyl-1)-pentanone-2.—α-(3-Methyl-6-pyridazonyl-1)-*n*-butyric acid (22 g., 0.11 mole) was mixed with acetic anhydride (100 ml.) and pyridine (100 ml.), and this mixture was heated at reflux until carbon dioxide evolution ceased (4 hr.). The reaction mixture was evaporated on a steam-bath at water pump vacuum and the residue was taken up in benzene (150 ml.). This solution was washed with 10% potassium carbonate solution (100 ml.) and the benzene layer was dried over anhydrous potassium carbonate. The benzene was distilled off under vacuum, and the residue was distilled, giving 13.5 g. (62% yield) of material boiling at 84° (0.05 mm.); $n^{25}D$ 1.5097.

3-Phenyl-6-pyridazonyl-1-acetone.—3-Phenyl-6-pyridazonyl-1-acetic acid (17.5 g., 0.075 mole) was mixed with acetic anhydride (75 ml.) and pyridine (75 ml.), and the mixture was heated at reflux for 4 hr. The volatile materials were removed by distillation under water pump vacuum from a steam-bath. The residue was semi-crystalline; after six consecutive crystallizations from ethanol, there was obtained 3.0 g. (17% yield) of pure ketone melting at 140–142°.

Anal. Calcd. for $C_{13}H_{12}N_2O_2$: C, 68.40; H, 5.30; N, 12.28. Found: C, 68.30; H, 5.18; N, 12.18.

1-(3-Methyl-6-pyridazonyl-1)-butanone-2.—3-Methyl-6-pyridazonyl-1-acetic acid (10.5 g., 0.062 mole) was mixed with propionic anhydride (70 ml.) and pyridine (70 ml.), and the mixture was heated at reflux until no more carbon dioxide was given off (20 hr.). The volatile materials were removed under water pump vacuum from a steam-bath, and the residue was taken up in benzene (100 ml.). This solution was washed with two 100-ml. portions of 1*N* sodium hydroxide solution, and the combined alkaline washes were washed with benzene (100 ml.) which was combined with the original organic layer. The benzene was evaporated from this solution leaving a crystalline residue which was recrystallized from petroleum ether (b.p. 60–70°) giving 5 g. (44% yield) of ketone melting at 95–97°.

4-(3-Methyl-6-pyridazonyl-1)-butanone-2.—A tiny chip of sodium was dissolved in absolute ethanol (100 ml.) and at 20° 3-methyl-6-pyridazone (11.0 g. 0.10 mole) and methyl vinyl ketone (8.0 g., 0.11 mole, freshly distilled at

120 mm.) were added to the ethanol. In the course of 15 minutes the temperature rose to 40° and after 1 hr. two drops of concentrated hydrochloric acid were added to neutralize the caustic. The ethanol was distilled off at water pump vacuum on the steam-bath, and the residue was distilled giving 17 g. (94% yield) of material boiling at 113–118° (0.05 mm.). This distillate crystallized almost immediately and melted at 57–59°; recrystallization from petroleum ether (b.p. 60–70°) did not raise this melting point.

Anal. Calcd. for $C_9H_{12}N_2O_2$: C, 60.43; H, 6.59; N, 15.55. Found: C, 60.15; H, 6.72; N, 15.35.

A small sample of this material was converted to the semicarbazone in the usual manner. After crystallization from water the semicarbazone melted at 179–180°.

Anal. Calcd. for $C_{10}H_{15}N_5O_2$: N, 29.55. Found: N, 29.0.

Alkylation of 3-Methyl-6-pyridazone with 3-Chloro-2-butanone.—To a solution of sodium (4.2 g., 0.18 atom) in ethanol (200 ml.) there was added 3-methyl-6-pyridazone (18.0 g., 0.16 mole); this mixture was cooled to below 10°, and 3-chloro-2-butanone (21.3 g., 0.18 mole) was added dropwise with good stirring. The mixture was heated at reflux for 2 hr. and then evaporated at water pump vacuum on the steam-bath. The residue was taken up in benzene (100 ml.), and this solution was washed with two 50-ml. portions of 30% potassium carbonate solution. The benzene solution was dried over anhydrous potassium carbonate and then evaporated under vacuum. The residue was distilled, giving 8.7 g. (30% yield) of material boiling at 90–100° (0.1 mm.), $n^{25}D$ 1.5063. $n^{25}D$ of 3-(3-methyl-6-pyridazonyl-1)-butanone-2 is 1.5226. Seeding this material with 4-(3-methyl-6-pyridazonyl-1)-butanone-2 did not induce crystallization.

A sample of this material was converted to a semicarbazone in the usual manner. The crude semicarbazone melted at 179–183°; after three recrystallizations from water it melted at 194–197°, no depression when mixed with the semicarbazone of 3-(3-methyl-6-pyridazonyl-1)-butanone-2. A mixed melting point with the semicarbazone of 4-(3-methyl-6-pyridazonyl-1)-butanone-2 melted at 160–175°.

Preparation of Alcohols. 3-(3-Methyl-6-pyridazonyl-1)-butanol-2.—A mixture of dry isopropyl alcohol (100 ml.), aluminum isopropoxide (freshly distilled) (20.4 g., 0.1 mole) and 3-(3-methyl-6-pyridazonyl-1)-butanone-2 was distilled very slowly for 2 hr. through a 10-in. helices packed column; 10 ml. of distillate was collected. The mixture was cooled and acidified with 25.5 ml. of concentrated hydrochloric acid and then made strongly alkaline with sodium hydroxide (24 g., 0.6 mole) dissolved in water (50 ml.). This solution was extracted with two 100-ml. portions of benzene, and the combined benzene extracts were dried over anhydrous potassium carbonate. The benzene was evaporated at water pump vacuum on the steam-bath and the residue was distilled, giving 15 g. (82.5% yield) of material boiling at 95° (0.1 mm.) which crystallized and melted at 73–76°.

Anal. Calcd. for $C_8H_{14}N_2O_2$: C, 59.32; H, 7.74; N, 15.38. Found: C, 59.16; H, 7.93; N, 15.26.

3-(3-Methyl-6-pyridazonyl-1)-2-methylbutanol-2.—Methylmagnesium iodide was prepared in the usual manner from magnesium turnings (2.64 g., 0.11 atom) and methyl iodide (17.1 g., 0.12 mole) in absolute ether (125 ml.). This solution was cooled to 10° and with good stirring there was added slowly a solution of 3-(3-methyl-6-pyridazonyl-1)-butanone-2 (16 g., 0.089 mole) in absolute ether (50 ml.); a solid complex was formed which was decomposed by the addition of ammonium chloride (7 g.) in water (20 ml.). An excess of anhydrous potassium carbonate was added to the mixture and the ether was separated and the solid was washed with two 100-ml. portions of benzene. The benzene and ether solutions were combined and evaporated at water pump vacuum on the steam-bath. The residue was distilled giving 12 g. of material boiling at 80–90° (0.02 mm.). This distillate was heated under reflux for 2 hr. with a solution of semicarbazide hydrochloride (6.9 g.) and anhydrous sodium acetate (4.9 g.) in water (30 ml.) in order to separate unreacted ketone as the semicarbazone. The resulting solution was evaporated to dryness under water pump vacuum on the steam-bath and the residue was treated with benzene (100 ml.), and this suspension was filtered. Evaporation of the benzene solution followed by distillation gave 7 g. (41% yield) of material boiling at 95–100° (0.02 mm.) which crystallized and melted at 53–55°. A small sample after recrystallization from petroleum ether (b.p. 60–70°) melted at 57.5–58.5°.

Anal. Calcd. for $C_{10}H_{18}N_2O_2$: C, 61.20; H, 8.22; N, 14.28. Found: C, 61.31; H, 8.08; N, 14.20.

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COMMUNICATIONS TO THE EDITOR

THE MECHANISM OF PEPSIN DENATURATION

Sir:

In 1930 Northrop[2] observed that the inactivation of pepsin was paralleled by the formation of acid insoluble pepsin. Subsequently Philpot[3] reported that the sedimentation constant (S) of pepsin showed a gradual decline between *p*H 5 and 11.

The gradual decline in sedimentation constant that Philpot observed has now been shown to represent the change from native to denatured pepsin and occurs over a very narrow range of *p*H, conforming to the enzyme kinetics of inactivation.[4] Single symmetrical boundaries, which spread at similar rates, were observed in the ultracentrifuge for both the native and denatured forms of pepsin. In 0.10 $\Gamma/2$ phosphate buffer the $S^{\circ}_{20,w}$ was 3.08 at *p*H 6.0 and 2.03 at *p*H 7.0 for the two forms, respectively.

To distinguish between frictional and mass changes in pepsin during inactivation, light scattering and viscosity methods were employed. At *p*H 6.42 the reduced intensity ($R = I_{90}r^2/I_0$) decreased uniformly to about 60% of its initial value. When the log of the fractional residual scatter $\{\log (R_t - R_\infty)/(R_0 - R_\infty)\}$ was plotted against time a linear relationship was observed. The kinetics agreed closely with that determined from the rate of formation of acid insoluble pepsin.

The viscosity of pepsin solutions was found to increase on inactivation. The rate of increase in viscosity paralleled the rate of enzyme inactivation; the data appear in Table I.

Since optical rotatory changes can serve as an indicator of molecular structural alterations in pro-

(1) Aided in part by grant No. C-1974 from the National Cancer Institute of the National Institutes of Health, Public Health Service, and by an Institutional Grant from the American Cancer Society.

(2) J. H. Northrop, *J. Gen. Physiol.*, **13**, 739 (1930).

(3) J. St. L. Philpot, *Biochem. J.*, **29**, 2458 (1935).

(4) J. Steinhardt, *Kgl. Danske Videnskab. Selskab Mat. fys. Medd.*, **14**, no. 11 (1937).