of 3-hydroxy-5,6-diphenyl-1-pyrazolo[b]pyrazine (VI, R = −H, R′ = $-C_6H_5$) with Raney nickel in boiling ethanol solution for three hours gave in 80% yield 2-amino-5,6-diphenylpyrazine-3-carboxamide (VII, R = −H, R′ = $-C_6H_5$), m.p. 203–205°, identical in all respects with an authentic sample prepared previously.[13] Similar cleavage of VI (R = $-CH_3$, R′ = −H) yielded 2-methylamino-pyrazine-3-carboxamide[7] (VII, R = $-CH_3$, R′ = −H, m.p. 200–201°. *Anal.* Calcd. for $C_6H_8ON_4$: C, 47.4; H, 5.3; N, 36.8. Found: C, 47.5; H, 5.3; N, 36.6). In a trial experiment, this reaction sequence leading to the pyrazine intermediates was shortened by several steps by direct condensation of 3-hydroxy-4-nitroso-5-aminopyrazole (V, R = −H) with biacetyl in ethanol solution in the presence of Raney nickel to give 2-amino-5,6-dimethyl-pyrazine-3-carboxamide (VII, R = −H, R′ = $-CH_3$, m.p. 255°. *Anal.* Calcd. for $C_7H_{10}ON_4$: C, 50.6; H, 6.1; N, 33.7. Found: C, 50.6; H, 6.1; N, 33.2) directly, the Raney nickel effecting both the reduction of the nitroso group and the ring cleavage of the subsequently formed pyrazolo[b]-pyrazine.

Since pteridines may be prepared directly from these intermediates by known methods,[1−5,7] the reactions outlined above constitute a new total synthetic approach to these important heterocycles.

(13) E. C. Taylor, THIS JOURNAL, **74**, 1651 (1952).

FRICK CHEMICAL LABORATORY
PRINCETON UNIVERSITY T. S. OSDENE
PRINCETON, NEW JERSEY E. C. TAYLOR

RECEIVED AUGUST 31, 1956

AN EXPLOSION DURING THE PREPARATION OF DIAZOACETONITRILE

Sir:

The preparation of diazoacetonitrile has recently been described[1] and we have repeated the procedure on several occasions without incident. However, a violent explosion occurred during one preparation and the operator was seriously injured as a result.

A concentrated solution of approximately 15 g. of diazoacetonitrile in methylene chloride was under water-pump vacuum in a four liter suction flask. The temperature was approximately 10° when the operator allowed air to enter the system. When the rubber stopper bearing the capillary was removed, the explosion resulted.

It is probable that some pure diazoacetonitrile was between the rubber stopper and the neck of the flask and the friction generated by removing the stopper was sufficient to initiate the explosion. *It is important that the nitrile be used only in dilute solution because it is highly explosive in concentrated form.*

A similar incident has been reported[1,2] during the attempted distillation of diazoacetonitrile but its highly explosive nature even in the presence of solvent has not been stressed adequately.

(1) S. H. Harper and K. C. Sleep, *J. Sci. Food Agr.*, **6**, 116 (1955).
(2) M. J. S. Dewar and R. Pettit, *J. Chem. Soc.*, 2026 (1956).

BAKER LABORATORY OF CHEMISTRY
CORNELL UNIVERSITY DONALD D. PHILLIPS
ITHACA, NEW YORK WILLIAM C. CHAMPION

RECEIVED SEPTEMBER 17, 1956

THE PREPARATION OF THE NEW COMPOUND CALCIUM COBALTATE(III)

Sir:

Although compounds of BaO and MgO with Co_2O_3 have been prepared as summarized by Mellor,[1] an attempt to prepare the compound $CaO{\cdot}Co_2O_3$ under similar conditions was not successful.

Difficulty in obtaining exact stoichiometry with cobalt compounds and the desirability of obtaining intimate mixture of reactants led to the following preparative procedure. Exactly one mole of cobaltous oxide which had been calcined at 950° under vacuum to insure absence of higher oxides was dissolved in hydrochloric acid and cobaltous hydroxide was precipitated with base. The precipitate was washed by decantation without loss until analysis showed absence of chloride ion in the wash water. Precipitated calcium carbonate (1.00 mole) was stirred into the cobaltous hydroxide, and the mixture was filtered. After drying at 110°, the mixture was calcined at 1100° for several hours in air. Analysis of the product corresponded to the formula $CaO{\cdot}Co_2O_{2.57}$.

A sample of 10.00 g. of $CaO{\cdot}Co_2O_{2.57}$ was put in a porcelain boat in a vacuum system at 525° in an atmosphere of oxygen. Reaction at this temperature was extremely slow, taking about three days to absorb sufficient oxygen to form a compound of formula $CaO{\cdot}Co_2O_3$.

A second 10.00-g. sample of the same material was allowed to react with oxygen at 660°. After 52 hours the pressure would decrease no further. Analysis agreed with the formula $CaO{\cdot}Co_2O_3$.

Analysis of Co(III) content of all samples was carried out by solution in hydroiodic acid and titration of the liberated iodine with thiosulfate.

Evidence of a probable chemical binding of calcium oxide in the compound is afforded by the observation that the calcium oxide in the compound could not be converted to soluble calcium hydroxide by placing in water at room temperature for 24 hours. The compound was slowly hydrolyzed by boiling 1 molar ammonium nitrate solution. Further, the compound would not absorb nitrogen dioxide from a dilute mixture with air in the temperature range 250 to 350° as does calcium oxide alone.

At room temperature the compound $CaO{\cdot}Co_2O_3$ has qualitatively the same magnetic properties as does Co_2O_3.

(1) J. W. Mellor, "A Comprehensive Treatise of Inorganic and Theoretical Chemistry," Longmans, Green & Co., New York, N. Y., 1935, vol. XIV, p. 594.

DEPARTMENT OF CHEMISTRY
AND CHEMICAL ENGINEERING
STANFORD UNIVERSITY JAMES D. RAY
CALIFORNIA RICHARD A. OGG, JR.

RECEIVED AUGUST 22, 1956

L-RIBULOSE-5-PHOSPHATE: FORMATION BY PURIFIED KINASE FROM *AEROBACTER AEROGENES*

Sir:

Lampen[1] has reported the enzymatic interconversion of L-arabinose ⇆ L-ribulose by extracts of

(1) J. O. Lampen, *Abstr. Proc. Amer. Chem. Soc.*, Sept., 1954, 44c-45c.

Lactobacillus pentosus, and has suggested that L-ribulose is phosphorylated in the presence of adenosine triphosphate (ATP). Similarly, Volk[2] tentatively identified heptulose, arabinose, ribose, and ribulose phosphates following incubation of L-arabinose and ATP with an extract of *Propionibacterium pentosaceum*.

Extracts of *Aerobacter aerogenes* also isomerize L-arabinose to L-ribulose. We now wish to report the finding of an enzyme that catalyzes the reaction

L-ribulose + ATP → L-ribulose-5-phosphate (L-Ru-5-P) + adenosine diphosphate (ADP)

This enzyme, L-ribulokinase, occurs in cells of *A. aerogenes* grown on L-arabinose, but not in significant amounts in cells grown on D-xylose. A 200-fold purified preparation is devoid of L-arabinose isomerase, phosphoketopentoepimerase[3] and transketolase,[4] but is contaminated with a small amount of phosphoriboisomerase.[5] Neither ADP nor UTP serve as phosphate donors.

The product of phosphorylation was prepared by incubating 0.25 mg. of purified enzyme (612 μM. phosphorylated per hr.), 1000 μM. ATP, 1000 μM, L-ribulose, 1000 μM. $MgCl_2$, 100 μM. sodium glutathione and 80 μM. Versene (20 ml. volume) at room temperature until alkali was no longer required to maintain the *p*H at 7.4. This was then chromatographed on a Dowex-1 formate column.[6] A very small peak identified as adenylic acid and a large symmetrical peak were located by the orcinol assay for pentoses.[6] The phosphorylated sugar was recovered (700 μM) as the alcohol insoluble barium salt.[6] The salt was redissolved and the barium precipitated as sulfate. The phosphate group was removed from an aliquot with acid phosphatase[7] and the solution deionized with a mixed bed of IR120(H^+) and IR45(OH^-).

In the carbazole method[8] the reaction with the free sugar was complete in 10 min., as is characteristic of ribulose.[9] Both the free and the phosphorylated sugars produced a red color with a maximum at 540 mμ. Both failed to give the carbazole color after incubation in 1 *N* NaOH for 20 min. as is characteristic of ribulose and D-ribulose-5-phosphate (D-Ru-5-P). In the orcinol method the ratio of absorbancy at 540 to 670 mμ for the free sugar was 0.80 as compared to 0.89 for L-ribulose. Paper chromatography of the free sugar revealed a single component having the same R_f and color reactions as ribulose[10] (Table I).

The phosphorylated product contained, per mole, 1 mole of organic phosphate. The rate of inorganic phosphate formation at 90° in 1 *N* H_2SO_4

TABLE I

CHROMATOGRAPHY OF THE DEPHOSPHORYLATED REACTION PRODUCT

	R_f		Color of Spot	
	Acetate-H_2O[a]	Phenol[b]	Orcinol-TCA[c]	Dimethyl-phenaline[c]
Xylose	0.14	0.51	Colorless	Brown
Fructose	.10	.56	Olive green[d]	Brown
Arabinose	.11	.57	olorless	Pink
Ribose	.19	.64	Colorless	Pink
Xylulose	.21	.65	Steel gray	Purple
Ribulose	.22	.68	Brownish gray	Rose[e]
Reaction product	.23	.68	Brownish gray	Rose[e]

[a] Three parts ethyl acetate, 1 part acetic acid, 3 parts water (upper phase). [b] Water saturated phenol. [c] As modified by M. I. Krichevsky, and W. A. Wood.[10] [d] Fluoresces green under ultraviolet. [e] Fluoresces orange under ultraviolet.

parallels that published for D-Ru-5-P.[6] The phosphorylated product resisted oxidation by bromine.[6,11] Two μM. of periodic acid were reduced[12] per μM. of phosphorylated product while under the same conditions 1.7 μM. were reduced per μM. of D-Ru-5-P.[13] $[\alpha]^{20}D$ +28° (c = 0.265 in 0.2 *N* HBr)[14] compared to $[\alpha]^{20}D$ −28.5° reported for D-Ru-5-P.[5,6] The phosphorylation product is there fore believed to be L-Ru-5-P.

In a system composed of crystalline glyceraldehyde-3-phosphate dehydrogenase from muscle and a purified preparation from spinach containing transketolase, epimerase and phosphopentoisomerase, diphosphopyridine nucleotide (DPN) was not reduced with L-Ru-5-P as the substrate although reduction was rapid with D-R-5-P. However, when an extract from *A. aerogenes* was added to the system, DPN was reduced with L-Ru-5-P. Thus, L-Ru-5-P appears to be the first phosphorylated intermediate in the metabolism of L-arabinose by *A. aerogenes*.

(2) W. A. Volk, *Fed. Proc.*, **15**, 1229 (1956).

(3) B. L. Horecker, J. Hurwitz and P. Z. Smyrniotis, THIS JOURNAL, **78**, 692 (1956).

(4) B. L. Horecker, P. Z. Smyrniotis and H. Klenow, *J. Biol. Chem.*, **205**, 661 (1953).

(5) J. Hurwitz, A. Weissbach, B. L. Horecker and P. Z. Smyrniotis, *ibid.*, **218**, 769 (1956).

(6) B. L. Horecker, P. Z. Smyrniotis and J. E. Seegmiller, *ibid.*, **193**, 383 (1951).

(7) R. M. Hochster, *Canad. J. Microbiol.*, **1**, 346 (1955).

(8) Z. Dische and E. Borenfreund, *J. Biol. Chem.*, **192**, 583 (1951).

(9) B. L. Horecker, J. Hurwitz and A. Weissbach, *ibid.*, **218**, 785 (1956).

(10) P. J. VanDemark and W. A. Wood, *J. Bacteriol.*, **71**, 385 (1956).

(11) S. Mitsuhashi and J. O. Lampen, *J. Biol. Chem.*, **204**, 1011 (1953).

(12) A. C. Neish, "Analytical Methods for Bacterial Fermentations," National Research Council, Ottawa, Ontario, 1952, pp. 20-31.

(13) Prepared from R-5-P with phosphoriboisomerase[5] by Dr. M. Wolin and Dr. W. Volk.

(14) Calculated from the pentose content based on a ribulose-5-PO_4:aldopentose ratio of 0.57 in the orcinol method.

(15) On educational leave of absence from Prairie Regional Laboratory, National Research Council, Saskatoon, Saskatchewan, Canada.

DEPARTMENT OF DAIRY SCIENCE
UNIVERSITY OF ILLINOIS
URBANA, ILLINOIS
F. J. SIMPSON[15]
W. A. WOOD

RECEIVED SEPTEMBER 12, 1956

THE *IN VITRO* SYNTHESIS OF 17α-HYDROXYPROGESTERONE AND Δ⁴-ANDROSTENE-3,17-DIONE FROM PROGESTERONE BY BOVINE OVARIAN TISSUE

Sir:

Almost 20 years after the suggestion was made[1] that exogenously administered testosterone might serve as a precursor for the estrogens, Baggett, *et al.*,[2] proved conclusively that human ovarian

(1) E. Steinach, H. Kun and O. Peczenik, *Wien. Klin., Wschr.*, **49**, 899 (1936).

(2) B. Baggett, L. L. Engel, K. Savard and R. I. Dorfman, *Fed. Proc.*, **14**, 175 (1955).