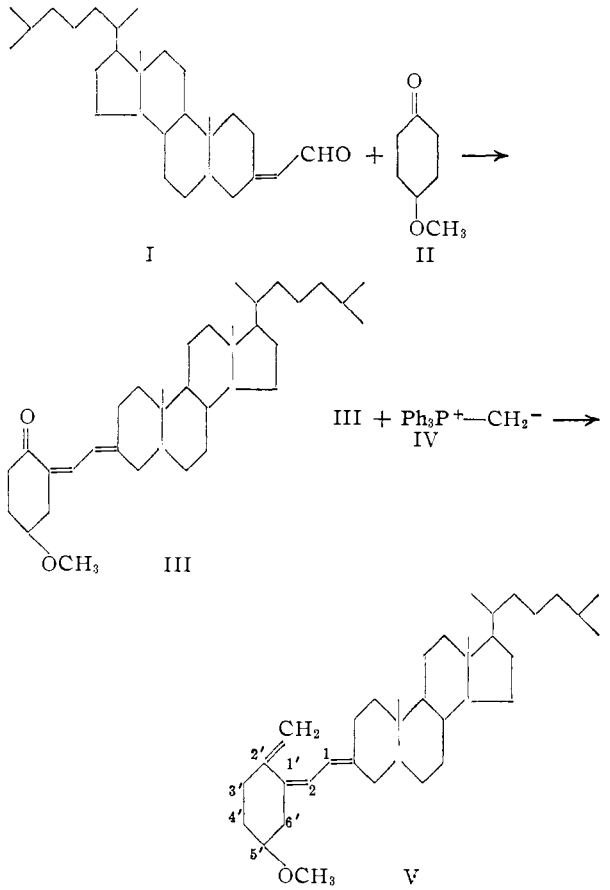
dene)-ethane (V), a homolog of vitamin D, which is biologically active. Following the scheme outlined in previous publication,[1] 7.54 g. of 2-cholestanylideneethan-1-al (I) [m.p. 114–115°. *Anal.* Calcd. for $C_{29}H_{48}O$: C, 84.39; H, 11.72. Found: C, 84.00; H, 11.82; ϵ (244.5 mμ), 30,250 (ethanol); $[\alpha]^{25}D$ −9.38° (chloroform)] was condensed in the dark with 4.7 g. of 4-methoxycyclohexanone (II) in a liter of *t*-butanol containing 4 g. of sodium hydroxide and 10 cc. of water. The mixture was stirred under nitrogen in the dark for 24 hr. A yellowish-brown amorphous product (7.4 g.) was isolated from this reaction and chromatographed in the dark using alumina (Act. III) from which was obtained two fractions: one



the 2,1′-*trans*-dienone (10%), m.p. 221–224° (dec.). *Anal.* Calcd. for $C_{36}H_{58}O_2$: C, 82.70; H, 11.18. Found: C, 82.73; H, 11.33; ϵ (309 mμ), 30,600 (ethanol); infrared α,β-conjugated >C=O band at 1674 cm.$^{-1}$; $[\alpha]^{25}D$, +86.8° (chloroform). Semicarbazone, m.p. 220–222°. *Anal.* Calcd. for $C_{37}H_{62}N_3O_2$: C, 76.50; H, 10.76; N, 7.23. Found: C, 76.72; H, 10.95; N, 6.94. The other fraction was the 2,1′-*cis*-dienone (12%), m.p. 186°. *Anal.* Calcd. for $C_{36}H_{58}O_2$: C, 82.70; H, 11.18; $\equiv$, 2.00. Found: C, 82.71; H, 11.07; $\equiv$, 1.85 (Pd.); ϵ (307 mμ), 12,200 (ether); infrared α,β-conjugated >C=O band at 1675 cm.$^{-1}$; $[\alpha]^{25}D$, +40.3° (chloroform).

Reaction of the 2,1′-*cis*-dienone (III) with triphenylphosphinemethylene (IV) in a pressure bottle gave an amorphous solid (23%), m.p. 193° (dec.); ϵ (265 mμ), 20,200 (ether). This was further purified by chromatography in a nitrogen atmosphere and in the dark giving again an amorphous product which had the following analyses.[2] *Anal.* Calcd. for $C_{37}H_{60}O$: C, 85.34; H, 11.61. Found: C, 84.22; 11.55; $[\alpha]^{25}D$, −10.6°; ϵ (267 mμ), 31,300 (ether); principal infrared bands[3] for $=CH_2$: 3100R, 1641W, 892S cm.$^{-1}$. Principal infrared bands for $=CH_2$ of vitamin D_3: 3100R, 1645M, 892M cm^{-1}.

An exploratory biological test of this homolog was carried out by Professor Robert S. Harris of the Nutritional Biochemical Laboratories of M.I.T. and he reports that the group of rachitic rats which was fed this homolog showed nearly the same degree of healing as the group which was fed vitamin D_2 at approximately the same concentration. These preliminary results do make it possible to estimate exactly the potency of the 2,1′-*cis*-homolog (V) but indicate quite definitely that its activity may approach that of vitamin D_2.

The 2,1′-*trans*-homolog was also synthesized from the 2,1′-*trans*-dienone and had an ϵ (272 mμ), 34,700 (ether). The *trans*-homolog also was found to be biologically active but very much less so than the *cis*-homolog. Details of this work will be published elsewhere.

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(1) N. A. Milas, L. C. Chiang, C. P. Priesing, A. A. Hyatt and J. Peters, THIS JOURNAL, **77**, 4180 (1955).

(2) This substance is highly sensitive to air oxidation and in spite of extensive precautions the carbon was always low.

(3) R = shoulder, W = weak, S = strong, M = medium.

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RECEIVED JUNE 6, 1957

A NEW SYNTHESIS OF THE CHROMIUM HEXACARBONYL

Sir:

We wish to report a new synthesis of chromium hexacarbonyl starting from easily available chromium compounds which appear to have been converted now for the first time, into the hexacarbonyl.

At present the only well described method for this synthesis is the "Grignard method" discovered by Job and Cassal[1] and substantially improved by Owen and co-workers.[2] Recently Fischer and Hafner[3] synthesized the carbonyl starting from chromium bis-cyclopentadienyl (no yield reported). Both these valuable methods suffer from some disadvantages such as the unsatisfactory over-all yields,[4] or the requirement of a rather

(1) A. Job and A. Cassal, *Compt. rend.*, **183**, 392 (1926).

(2) B. B. Owen, J. English, Jr., H. G. Cassidy and C. Vanderbilt Dundon, THIS JOURNAL, **69**, 1723 (1947).

(3) E. O. Fischer and W. Hafner, *Z. Naturf.*, **10b**, 140 (1955).

(4) W. H. Cumming, J. A. Horn and P. O. Ritchie, *J. Appl. Chem.*, **2**, 624 (1952).

involved procedure for preparing the suitable starting material.

We have now observed[5] that chromic acetylacetonate, chromic and chromous salts of organic acids such as acetic and 2-ethylhexanoic can be reduced easily and converted into the hexacarbonyl under high pressure of carbon monoxide if pyridine or related bases are employed as a reaction medium.

Our method consists in dissolving (or suspending) any one of the above compounds in pyridine, containing catalytic amounts of halogens or halogenated substances. The mixture is then treated at 80–170° with an excess of powdered magnesium or zinc and 100–300 atm. of carbon monoxide. When soluble chromium compounds are employed the yields are as high as 80–90%.

We believe the base to play an essential role in the synthesis because intermediate pyridine-containing complexes are formed in the course of the reaction.

It is also noteworthy that the hexacarbonyl as such is still present in substantial quantities in the end-products of the reaction. This seems not to happen in the synthesis by the Grignard method, the hexacarbonyl, in this case, being produced uniquely after the hydrolysis of the reaction mixtures.[4]

Experimental.—Chromium acetylacetonate (17.5 g.), magnesium (4.5 g.) and 80 g. of a 2% solution of iodine in dry pyridine are charged in a stainless steel oscillating autoclave of 500-ml. capacity. After removal of air, oscillation is started and pure oxygen-free carbon monoxide is compressed into the vessel up to a pressure of 190 atm. The autoclave is then warmed to 160° within one hour and maintained at this temperature (± 2°) for six hours, while pressure drops from 325 to 285 atm. After cooling and venting, the hexacarbonyl and the other reaction products are quantitatively transferred into a 3-liter two-necked distillation flask with the aid of a large amount of water. On distillation of the mixture, the white hexacarbonyl is drained off. The distillate is filtered, washed with chilled methanol and paper dried. Sublimation of the powder at 70–75° at 15 mm. affords 9.0 g. of pure chromium hexacarbonyl in coarse crystals (yield 82%).

(5) Italian Patent Application No. 671, February 4, 1957 (to Montecatini S.p.A.).

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RECEIVED MAY 4, 1957

PHOSPHATE TRANSFER CATALYZED BY PHOSPHOGLYCERIC ACID MUTASE

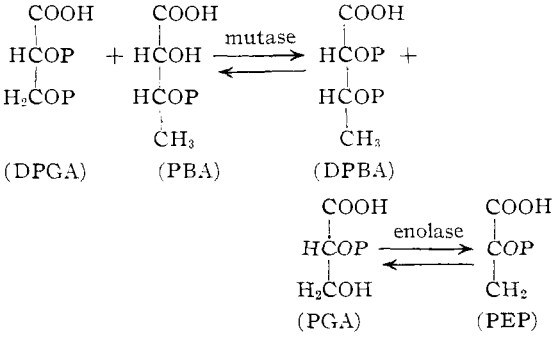
Sir:

The activation of partially purified muscle phosphoglyceric acid mutase by diphosphoglyceric acid (DPGA) was reported by Sutherland, *et al.*,[1] and we have undertaken the further study of this process with the crystalline enzyme, which was obtained from purified muscle enzyme[2] by employing the conditions found by Rodwell, *et al.*,[3] for the crystallization of the yeast mutase. In order to eliminate the possibility of DPGA contamination of the monophosphate substrate, synthetic phosphoenolpyruvate (PEP)[4] recrystallized as the cyclohexylamine salt[5] was used, and 2-phosphoglyceric acid (2PGA) was generated from it with crystalline enolase. Mutase activity was detected by its effect on the absorbance at 240 mμ of the equilibrated mixture, (PEP $\overset{\text{enolase}}{\rightleftharpoons}$ 2PGA $\overset{\text{mutase}}{\rightleftharpoons}$ 3PGA).

Synthetic DPGA[6] activated the mutase over the range 10^{-4} to 10^{-7} M, and the K_m for the conditions used was 2×10^{-6} M. The enzyme also exhibited activity under the same conditions when no DPGA was added. This activity was proportional to the amount of enzyme added and corresponded to 5% of the activity displayed when the enzyme was saturated with DPGA. The same order of activity was observed when the substrate was synthetic 3PGA,[7] although the presence of DPGA in trace amounts is here possible. In the latter case the activity was followed by the formation of PEP.

These results are indicative of two forms of the mutase, one which requires DPGA activation and one which does not. The enzyme, like phosphoglucomutase,[8] probably exists in a nonphosphorylated and a phosphorylated form. The latter species could exhibit activity in the absence of DPGA, and DPGA would activate by donating one of its phosphate groups to the nonphosphorylated form.

In experiments testing the specificity of DPGA as a donor of phosphate groups, we made use of the *erythro*-2,3-dihydroxybutyric acid monophosphates (DPBA)[9]; which have been shown to be active substrates for the mutase[2] but not for enolase.[5] The acceptance of phosphate from DPGA by PBA results in the formation of a mixture of the phosphoglyceric acid isomers (2PGA and 3PGA) which



are in part converted to PEP if enolase is present.

(1) E. W. Sutherland, T. Posternak and C. F. Cori, *J. Biol. Chem.*, **181**, 153 (1949).

(2) R. W. Cowgill and L. I. Pizer, *ibid.*, **223**, 885 (1956).

(3) V. W. Rodwell, J. C. Towne, and S. Grisolia, *Biochim. et Biophys. Acta*, **20**, 394 (1956).

(4) E. Baer and H. O. L. Fischer, *J. Biol. Chem.*, **180**, 145 (1949).

(5) F. Wold and C. E. Ballou, *ibid.*, in press.

(6) E. Baer, *ibid.*, **185**, 673 (1950).

(7) C. E. Ballou and H. O. L. Fischer, Abstracts of Papers, 126th Meeting, American Chemical Society, 7–D, 1954.

(8) V. A. Najjar and M. E. Pullman, *Science*, **119**, 631 (1954).

(9) C. E. Ballou, THIS JOURNAL, **79**, 984 (1957).