space group $P2_12_12_1$ with four molecules (K or Rb)-$C_6O_6H_5$ in cells of dimensions:

	a (Å.)	b (Å.)	c (Å.)	Cell volume (Å.^{3})	Density (g. cm.$^{-3}$) obs.	calcd.
K	9.059	12.681	6.640	762.8	1.838	1.848
Rb	9.190	12.639	6.825	792.7	2.142	2.166

The standard error of the cell dimensions is about ±0.15% and that of the observed densities about ±0.5%.

The structure was determined from projections on the three principal planes using the method of isomorphous replacement. The three projections for the potassium salt have been refined by difference Fourier maps and then by least squares using the full matrix to account for the overlap of atoms. At the present stage of refinement the R values for the $hk0$, $0kl$, and $h0l$ projections are 7.6, 11.5, 12.6%, respectively, the unobserved reflections being included at one-half their estimated upper limit.

The structure which we have obtained for the lactone ion confirms the conclusion of Gawron and Glaid that the carboxyl groups are *cis* with respect to the lactone ring. We also find that the metal atom is coördinated with eight oxygen atoms. All oxygen atoms including that in the lactone ring take part in this coördination. The structure clearly explains the pronounced cleavage of these crystals parallel to the b face.

Complete details of the structure analysis and a discussion of the bond lengths and bond angles will be presented elsewhere. We also hope to determine the absolute configuration of the rubidium salt by the method of Bijvoet and his co-workers.[4]

(4) J. M. Bijvoet, A. F. Peerdeman and A. J. van Bommel, *Nature* **168**, 271 (1951).

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REVERSIBILITY OF AMINO ACID INCORPORATION INTO RIBONUCLEIC ACID

Sir:

The incorporation of C^{14}-labeled amino acids into ribonucleic acid[1] has been reported[2] and confirmed.[3] That a specific amino acid-activating enzyme is required for the incorporation into ribonucleic acid me-amino of the amino acid it activates,[3a,b] suggests these reactions

$$\text{Enzyme} + \text{amino acid} + \text{ATP} \rightleftarrows [\text{enzyme-amino acyl-AMP}] + \text{P-P} \quad (1)$$

$$[\text{Enzy acyl-AMP}] + \text{RNA} \rightleftarrows \text{amino acid-RNA} + \text{enzyme} + \text{AMP} \quad (2)$$

(1) These abbreviations are used: RNA, ribonucleic acid; AMP, adenosine 5′-monophosphate; ATP, adenosine 5′-triphosphate; ADP, adenosine 5′-diphosphate; TCA, trichloroacetic acid; GMP, guanosine 5′-monophosphate; CMP, cytidine 5′-monophosphate; P-P, pyrophosphate.

(2) M. B. Hoagland, P. C. Zamecnik and M. L. Stephenson, *Biochim. et Biophys. Acta*, **24**, 215 (1957).

(3) (a) R. S. Schweet, F. C. Bovard, E. H. Allen and E. Glassman, *Proc. Natl. Acad. Sci.*, **44**, 173 (1958); (b) P. Berg and E. J. Ofengand, *ibid.*, **44**, 78 (1958); (c) K. Ogata and H. Nohara, *Biochim. et Biophys. Acta*, **25**, 659 (1957).

The reversibility of Reaction 1 has been shown previously.[4] Indirect evidence suggesting reversal of the overall reaction has been reported.[5] Reversal has been followed directly here by measuring the cleavage of isolated amino acid-RNA and incorporation of AMP into ATP.

Cleavage of isolated threonine-RNA and leucine-RNA, and incorporation of C^{14}-labeled AMP into ATP, dependent on amino acid-RNA, is shown in Table I. No ATP or free amino acid was added in these experiments. Both amino acid-RNA compounds were cleaved in the presence of AMP plus P–P (complete mixture), although the quantitative importance of P–P depends on the particular amino-acid-RNA and enzyme fraction used. In other experiments, the amount of threonine-RNA split depended on the amount of AMP added, GMP or CMP did not replace AMP; Mg ion was essential; and leucine-RNA was not split by threonine-activating enzyme.

TABLE I

CLEAVAGE OF AMINO ACID-RNA AND INCORPORATION OF AMP INTO ATP

Constituents	Starting with threonine-RNA: Counts/min. of C^{14}-AMP found in ATP	Starting with threonine-RNA: Counts/min. of C^{14}-threonine-RNA remaining	Starting with leucine-RNA: Counts/min. of C^{14}-AMP found in ATP	Starting with leucine-RNA: Counts/min. of C^{14}-leucine RNA remaining
(1) Complete mixture[a]	408	168	526	416
(2) Zero time control	114	983	84	860
(3) As (1), but RNA in place of amino acid-RNA[b]	108	...	64	...
(4) As (1), but boiled enzyme	100	887	...	...
(5) As (1), but P–Pomitted	128	316	100	820
(6) As (1), but AMP omitted	...	805	...	804

[a] The complete reaction mixture for AMP incorporation into ATP contained 0.5 ml. of activating enzyme, approximately 0.5 mg. of RNA or C^{12}-amino acid-RNA; 100 μmoles of Tris buffer, *p*H 7.5; 2 μmoles of C^{14}-AMP (Schwarz Laboratories), containing 60,000 counts/min./μmole; 2 μmoles of magnesium chloride; 2 μmoles of P–P; and water to make 1.8 ml. Mixtures were incubated at 37° for 15 minutes; then 0.7 mg. of casein, 1.8 ml. of 7% TCA and 10 μmoles of ATP were added and the ATP isolated and counted (see Holley[5]). Incubation conditions for studying cleavage of amino acid-RNA were similar, but using C^{12}-AMP and C^{14}-amino acid-RNA, and counting residual amino acid-RNA (precipitated by perchloric acid). Separate activating enzyme fractions for leucine or threonine activation, free of RNA, were prepared from guinea pig liver.[6] Labeled amino acid-RNA compounds were prepared by phenol extraction from the usual reaction mixture for incorporation,[3a] and contained 1 mμmole of C^{14}-amino acid (2500 c.p.m.) per mg. of RNA. [b] RNA was prepared by phenol extraction and was active for amino acid incorporation.[7] In this experiment 0.5 mμmole of C^{12}-threonine or leucine was added. The dash indicates "experiment omitted."

(4) M. B. Hoagland, E. B. Keller and P. C. Zamecnik, *J. Biol. Chem.*, **218**, 345 (1956); J. A. De Moss, S. M. Genuth and G. D. Novelli, *Proc. Natl. Acad. Sci.*, **42**, 325 (1956); P. Berg, *Fed. Proc.*, **16**, 152 (1957).

(5) R. W. Holley, THIS JOURNAL, **79**, 658 (1957); M. B. Hoagland, M. L. Stephenson, J. F. Scott, L. I. Hecht and P. C. Zamecnik, *J. Biol. Chem.*, **231**, 241 (1958); J. Mager and F. Lipmann, *Proc. Natl. Acad. Sci.*, **44**, 305 (1958).

(6) E. H. Allen, E. Glassman and R. S. Schweet, in preparation.

(7) K. S. Kirby, *Biochem. J.*, **64**, 405 (1956).

Incorporation of C^{14}-labeled AMP into ATP is the most direct evidence for reversibility of amino acid incorporation into RNA. No incorporation of AMP into ATP occurred when RNA plus free amino acid was used in place of amino acid-RNA, or when P–P was omitted. Calculation of the data shows that the molar rate of AMP incorporation into ATP greatly exceeds the rate of amino acid-RNA breakdown, suggesting that reactions which form unlabeled ATP occur during the incubation.

The approximate equilibrium position of these reactions was near unity, indicating the high energy nature of the amino acid-RNA linkage.

(8) Work done during the tenure of an Established Investigatorship of the American Heart Association. These studies were supported by a grant from the National Science Foundation.

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16-ALKYLATED CORTICOIDS. I. 16α-METHYLPREDNISONE AND 16β-METHYLPREDNISONE[1]

Sir:

The recent publication by Boland[2] on 16α-methyl corticosteroids prompts us to report our studies of both 16α- and 16β-methyl steroids which possess a high order of anti-inflammatory activity without salt retention in animal and clinical trials.

Reaction of 3α-acetoxy-16-pregnene-11,20-dione[3] (I) with diazomethane gave an intermediate pyrazoline,[4] m.p. 199–200° dec., $[\alpha]_D$ +149.6° (all rotations 1% in diox.). *Anal.* Found: C, 69.51; H, 7.98; N, 6.69. Pyrolysis of this product at its melting point gave 3α-acetoxy-16-methyl-16-pregnene-11,20-dione, m.p. 163–166°, $[\alpha]_D$ +69.9°, λ_{max}^{MeOH} 248 mμ (ε 10,800). *Anal.* Found: C, 74.58; H, 8.55. Reduction with palladium yielded 3α-acetoxy-16β-methyl pregnane-11,20-dione (II) m.p. 160–163°, $[\alpha]_D$ +93.6°, no ultraviolet absorption between 220–300 mμ. *Anal.* Found: C, 74.37; H, 9.06. Enol acetylation with *p*-toluenesulfonic acid and acetic anhydride, then treatment with peracetic acid and finally alkaline hydrolysis, gave 3α,17α-dihydroxy-16β-methylpregnane-11,20-dione, m.p. 182–185°, $[\alpha]_D$ +83.6°. *Anal.* Found: C, 72.82; H, 8.92. Bromination at C-21 and then treatment with potassium acetate gave 3α,17α-21-trihydroxy-16β-methylpregnane-11,20-dione 21-acetate, m.p. 200–206°, $[\alpha]_D$ +119.6°. *Anal.* Found: C, 68.79; H, 8.39. Oxidation with N-bromosuccinimide produced the 3-ketone, m.p. 198–202°, $[\alpha]_D$ +128.0°. *Anal.* Found: C, 69.04; H, 8.10. Dibromination at positions 2 and 4, followed by dehydrobromination with dimethylformamide, produced 16β-methylprednisone 21-acetate (III) m.p. 232–235°, $[\alpha]_D$ +213.6°, λ_{max}^{MeOH} 238 mμ (ε 14,200). *Anal.* Found: C, 69.24, H, 7.21. Hydrolysis with potassium bicarbonate gave 16β-methylprednisone, m.p. 210–204°, $[\alpha]_D$ +190.2°, λ_{max}^{MeOH} 238 mμ (ε 14,700). *Anal.* Found: C, 71.19; H, 7.37

Reaction of I with methylmagnesium iodide produced 3α-hydroxy-16α-methylpregnane-11,20-dione,[5] m.p. 149–154°, $[\alpha]_D$ +100.5°, no selective absorption between 200 and 340 mμ. *Anal.* Found: C, 74.16; H, 9.41. This was converted into 16α-methylprednisone 21-acetate (m.p. 212–214° $[\alpha]_D$ +157.8°, λ_{max}^{MeOH} 238 mμ (ε 15,500). *Anal.* Found: C, 69.84; H, 7.22) by the same procedure used for the conversion of II to III (*i.e.*, enol acetylation and peroxidation, 21-bromination and acetoxylation, oxidation at C-3 and 2,4-dibromination and dehydrobromination).

A direct comparison of prednisone and its 16-methyl derivatives in human subjects utilizing (1) metabolic balance studies[6] consisting of the analysis of diet, urine and feces for calcium, phosphorus, nitrogen, sodium and potassium, and (2) the clinical response of patients indicate that 16-methylation (α or β) of the parent steroid, prednisone, is associated with an enhancement of anti-anabolic properties and an increase of 30–50% in both anti-inflammatory and sodium excreting properties. Unlike 16α-hydroxylation, 16α- or 16β-methylation contributes to anti-inflammatory potentiation. The *in vivo* conversion of the 16-methyl corticoids into urinary 17-keto steroids is limited to less than 5%: a conversion slightly less than that obtained with the parent steroids unsubstituted at position 16, and much less than that obtained with cortisone or hydrocortisone.

(1) After submission of this manuscript, a Communication appeared [G. Arth, D. Johnston, J. Fried, W. Spooncer, D. Hoff and L. Sarett, THIS JOURNAL, **80**, 3160 (1958)] describing the preparation of 16α-methylprednisone by essentially the same route. We have tried to eliminate as much of the common material as possible.

(2) E. W. Boland, *Cal. Med.*, **88**, 417 (1958).

(3) H. Slates and N. Wendler, *J. Org. Chem.*, **22**, 498 (1957).

(4) *Cf.* A. Wettstein, *Helv. Chim. Acta*, **27**, 1803 (1944).

(5) *Cf.* R. Marker and H. Crooks, THIS JOURNAL, **64**, 1280 (1942).

(6) E. C. Reifenstein, F. Albright and S. Wells, *J. Clin. Endocrinol.*, **5**, 367 (1945).

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THE INTERMEDIATE COBALT HYDROCARBONYL–OLEFIN COMPLEX IN THE OXO REACTION[1]

Sir:

The several mechanisms[2,3,4] which have been suggested for the oxo synthesis all involve a rate-determining displacement of a mole of carbon monoxide from a carbonyl of cobalt by the attacking olefin. The present study shows that not only does complexing occur between olefin and hydrocarbonyl[5] under room conditions without the libera-

(1) We wish to thank the Houdry Process Corp. for a generous fellowship which made this work possible.

(2) H. W. Sternberg, R. Markby and I. Wender, THIS JOURNAL, **79**, 6116 (1957); I. Wender and M. Orchin, in "Catalysis," Vol. V, Reinhold Publishing Corp., New York, N. Y. 1957, p. 124.

(3) A. R. Martin, *Chem. and Ind.*, 1536 (1954).

(4) G. Natta, R. Ercoli, S. Castellano and P. H. Barbieri, THIS JOURNAL, **76**, 4094 (1954).

(5) M. Orchin, L. Kirch and I. Goldfarb, *ibid.*, **78**, 5450 (1956).