reported by Ariya *et al.*[4] The observed density is in good agreement with the value of 4.89 g/cm³ calculated for a cell content of two formula units of CrO_2.

The axial ratio of chromium dioxide ($c/a = 0.660$) corresponds to a relatively long distance between adjacent Cr atoms and indicates that no attracting forces are acting between these metal atoms in contrast to what is the case in several other compounds of transitions metals with valency states lower than their maximum ones [8]. Of particular interest in this connection is the rutile type phase $(Cr, Mo)O_2$ with its considerably lower values of c/a (down to 0.60) which have been interpreted as caused by the presence of metalmetal bonding [9].

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1. Michel, A. and Bénard, J. *Compt. rend.* **200** (1935) 1316.
2. Michel, A. and Bénard, J. *Bull. soc. chim. France* **10** (1943) 315.
3. Schwartz, R. S., Fankuchen, I. and Ward, R. *J. Am. Chem. Soc.* **74** (1952) 1676.
4. Ariya, S. M., Shchukarev, S. A. and Glushkova, V. B. *Zhur. Obshchei Khim.* **23** (1953) 1241—5.
5. Glemser, O., Hauschild, U. and Trüpel, F. *Z. anorg. u. allgem. Chem.* **277** (1954) 113.
6. Thamer, B. J., Douglass, R. M. and Staritzky, E. *J. Am. Chem. Soc.* **79** (1957) 547.
7. Bhatnagar, S. S., Prakash, B. and Hamid, A. *J. Chem. Soc. 1938, 1428.*
8. Marinder, B.-O. and Magnéli, A. *Acta Chem. Scand.* **11** (1957) 1635.
9. Sundholm, A., Andersson, S., Magnéli, A. and Marinder, B.-O. *To be published.*

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Chemical Synthesis of Oleyl Adenylate

BENGT BORGSTRÖM

Department of Physiological Chemistry, University of Lund, Lund, Sweden

Mixed anhydrides of adenylic acid with short and middle chain fatty acids have been proposed as intermediates in the enzymatic activation of these acids to their CoA derivatives [1-5]. Chemical synthesis of these adenylates has been reported using three different methods: 1) coupling of silver adenylate with the acid chloride (acetyladenylate [1]), 2) the reaction of the acid anhydride with adenylic acid in aqueous pyridine (buturyl and hexanoyl adenylate [2,5]), and 3) the condensation of fatty acid and adenylic acid in the presence of carbodiimides (buturyl adenylate [5]).

For an attempted synthesis of long chain fatty acid adenylates method 2 did not seem practical due to the low solubility of the long chain anhydrides. Of the two other methods the carbodiimide method was found to be the most practical and to give the best yields.

This paper will describe the synthesis of oleyl adenylate by the condensation of oleic acid with adenylic acid in the presence of di*cyclo*hexylcarbodiimide and also includes experiments to show the metabolic activity of this compound with rat liver mitochondria *in vitro*.

EXPERIMENTAL

Materials. Adenylic acid (AMP) was obtained from Sigma Chemical Company and di*cyclo*hexylcarbodiimide (DCC) from Fluka A.G.

Methods. Oleyl adenylate was estimated by the hydroxamic acid-$FeCl_3$ method modified for the acid insoluble hydroxamates of oleic acid [6]. A sample containing approximately 1 mg adenylate in 0.1—0.5 ml was added with 1 ml of a freshly prepared solution of equal volumes of 28 % hydroxylamine and 14 % sodium hydroxide. After 5 min 3 ml of 7 % perchloric acid was added and the insoluble hydroxamates filtered off on a paper filter (Munktell No. 8, diam. 5.5 cm) and washed on the filter two times with each 3 ml of the perchloric acid solution. The hydroxamates were then extracted from the filter with 3 ml of a dilution of Hill's reagent 1:20 in ethanol and the extinction of the solution measured at 530 mμ. With this method 1 μmole hydroxamate gives an extinction of 0.28, the molecular extinction of hydroxamates being around 1 000.

Synthesis of oleyl adenylate. 1 g adenylic acid (= 2.74 mmole) was dissolved in 40 ml pyridine and 8 ml water and 10 ml oleic acid (= 30 mmole) added. This solution was cooled to 0° and was added to an icecold solution of 10 g DCC in 20 ml pyridine. The reaction mixture was allowed to stand for 6 h in an ice-bath and was then added with 32 ml ice-cold

solution containing 66 mg lithium hydroxide (= 2.74 mmole). After 5 min the di-*cyclo*hexylurea was filtered off on a glassfilter and the clear solution extracted with 3 times 200 ml ether to remove excess oleic acid and pyridine. The water phase containing the adenylate was lyophilized and 1.40 g of a slightly yellow powder obtained. When assayed with the hydroxamic acid method this powder contained 1.26 μmoles adenylate per mg corresponding to 0.80 mg oleyl adenylate. The yield was 64 %, calculated on the AMP, and the purity around 80 %. This preparation was further purified by chromatography on a column of Whatman No. 1 paper powder (coarse grade) using *iso*propanol:water 70:30 as solvent [5]. For the purification of 200 mg of the crude preparation a column was prepared from 90 g paper powder (diam. 34 cm). The column was washed with *iso*propanol:water until the effluent was free from substances adsorbing at 259 mμ. 200 mg of the adenylate mixture was dissolved in 10 ml of the 70 % *iso*propanol and applied to the column which was developed with the same solvent. 20 ml fractions were collected and their optical density at 259 mμ determined. A pea kwas obtained between 160 and 240 ml of effluent. These 80 ml were added with 48 ml water and 130 ml ether to extract the *iso*propanol from the water phase and the latter lyophilized. The ultraviolet adsorption spectrum of a solution of this powder showed a single peak at 259 mμ with an optical density at this wavelength of 23.0 per mg as compared to a theoretical figure of 24.4 assuming the oleyl adenylate to have the same molar extinction coefficient as AMP (= 15.4×10^3). The ratio between activated fatty acid as determined by the hydroxamic acid method and the AMP content as derived from the optical density at 259 mμ was 0.96.

A study of the effect of fatty acid to AMP ratio on the yield of adenylate showed that 1, 3 and 9 moles of oleic acid per mole of AMP gave a yield of 45, 69 and 82 %, respectively, of adenylate calculated on the AMP of the reaction. To get the best yield calculated on the fatty acid moiety, as desired in the synthesis of fatty acid labeled adenylate, therefore equimolar amount of AMP and fatty acid should be used.

The method described gave low yields for the synthesis of adenylates with palmitic and stearic acid due to the low solubility of these acids in the solvent mixture used at 0°. If the reaction was allowed to take place at room temperature to facilitate the solution of these acids, the yield of hydroxamic acid forming substances was low most probably due to the formation of polyacylated compounds [5].

Table 1. Oxidation of oleyl adenylate and oleic acid with rat liver mitochondria. The Warburg vessels contained final concentration of 0.01 M phosphate buffer pH 7.4, 0.05 M KCl, 0.005 M $MgCl_2$, 0.0005 M succinate as priming substrate and 10^{-5} M cytochrome c. Mitochondria added in 0.15 M KCl suspension corresponding to 1.5 g liver. Additions according to table. Final volume 2.0 ml; gas phase air. Incubated at 30°: equilibration time 10 min.

Additions	O_2 uptake, μl, 0—20 min.
None	12.8
Oleyl adenylate 0.25 μmole	54.8
» » 0.50 »	58.9
» » 1.0 »	31.6
» » 2.0 »	11.1
ATP 2 μmole	32.8
» 2 + oleic acid 0.25 μmole	65.9
» 2 » » 0.50 »	—
» 2 » » 1.0 »	60.3
» 2 » » 2.0 »	7.2

From Table 1 it appears that rat liver mitochondria can oxidize oleyl adenylate at the same rate as oleic acid in the presence of adenosine triphosphate (ATP) and thus can replace the ATP requirement for the oxidation of oleic acid [7]. The optimal concentration for the oxidation of adenylate in this system is the same as for oleic acid, *i.e.* 2.5×10^{-4} M. At higher substrate concentration both adenylate and oleic acid inhibit respiration.

1. Berg, P. *J. Biol. Chem.* **222** (1956) 1010.
2. Lee Peng, C. H. *Biochim. et Biophys. Acta* **22** (1956) 42.
3. Whitehouse, M., Moeksi, H. and Gurin, S. *J. Biol. Chem.* **226** (1957) 813.
4. Jencks, W. P. and Lipmann, F. *J. Biol. Chem.* **225** (1957) 207.
5. Talbert, P. T. and Huennekens, F. M. *J. Am. Chem. Soc.* **78** (1956) 4671.
6. Kornberg, A. and Pricer, W. E. *J. Biol. Chem.* **204** (1953) 329.
7. Kennedy, E. P. and Lehninger, A. L. *J. Biol. Chem.* **185** (1950) 275.

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