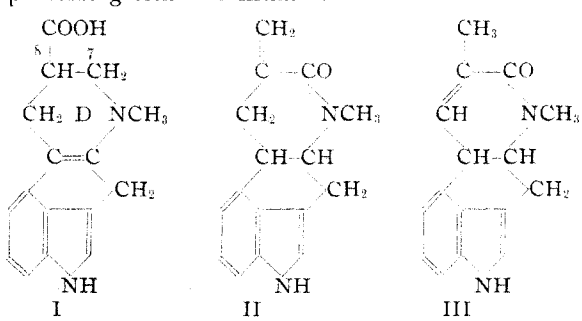
In a communication by L. C. Craig, T. Shedlovsky, R. G. Gould, Jr., and W. A. Jacobs which is now in press, an investigation of the dissociation constants of lysergic acid and its derivatives has been reported, which has made it necesssary to revise our previous tentative conclusion that the COOH group of lysergic acid is in position 7, *i. e.*, on the carbon atom adjoining the N atom of Ring D. Such an arrangement would place it in the category of a substituted α-amino acid like proline. Instead, the evidence indicates a β-amino acid derivative and that the point of attachment of the COOH group is the carbon atom β to the N atom or position 8 as shown in formula I.

The formation of the above neutral pyrolysis product from dihydrolysergic acid fits in well with such β-substitution of the COOH group and must be interpreted as an unsaturated cyclic amide possessing either formula II or III.



I II III

It is well known that β-amino acids, in contradistinction to the α-acids, decompose readily into unsaturated acids and ammonia. For instance, β-alanine gives readily ammonia and acrylic acid. In accordance with this property, dihydrolysergic acid should cleave between the N atom and carbon atom 7 to give an unsaturated complex secondary amino acid which would at once lactamize. Model experiments with the various piperidine carboxylic acids are now in progress to verify the nature and formation of this pyrolysis product of dihydrolysergic acid.

THE LABORATORIES OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH
NEW YORK, N. Y.

WALTER A. JACOBS
LYMAN C. CRAIG

RECEIVED JUNE 21, 1938

ENOL ETHERS OF STEROID KETONES

Sir:

The following is a report of the synthesis of enolic ethers of unsaturated ketonic sterols. For the preparation of these compounds the modification of Arbuzov and Mikhailova [*J. Gen. Chem.* (*U. S. S. R.*), **6**, 390 (1936)] of the classical Claisen method was used. Instead of obtaining the expected ketal, the reaction yielded directly the enol ether, one molecule of alcohol having been split out.

As a class, these compounds are stable in a solid state, but quite unstable in solution. From Table I it may be seen that in chloroform solution at room temperature the rotation changes rapidly from a negative to a positive one, the final rotation in each case being that of the free ketone. Very probably the traces of hydrochloric acid in the chloroform are the cause of the rapid splitting of the ethoxyl group, since in pyridine solution the rotation remained practically unchanged. By hydrolyzing the testosterone enol ether propionate with alcoholic potash it was possible to remove the propionic acid group without splitting the ethoxyl out of the molecule.

TABLE I

Time, hours	Specific rotation of enol ethyl ethers: Cholestenone[a]	Testosterone benzoate[b]	Testosterone propionate[c]
0	−102.3	− 42.5	−140
0.5	− 33.8	+130	−114
1	+ 18.2	+142	
2	+ 67.6	+143	+60
24	+ 87.7		+88 (48 hours)

[a] 1.1% in chloroform. [b] 1.01% in chloroform. [c] 1.2% in chloroform.

In pyridine solution the specific rotations were −96.0° for cholestenone enol ethyl ether, and −67.5° for testosterone benzoate enol ethyl ether. These values changed only slightly after standing for several hours.

The fact that all the new substances show initially a negative rotation would indicate that one of the double bonds is in ring A, the other in ring B [Ulrich Westphal, *Ber.*, **70**, 2128 (1937)].

These substances are prepared by dissolving 5 g. of the ketone in 10 cc. of ethyl orthoformate and 1 cc. of formic acid. The solution is cooled to 20° and one drop of concentrated sulfuric acid added. The mixture is warmed on the water-bath to 50° with constant stirring, held at this temperature for ten minutes. After standing overnight at room temperature, ether is added and the solution washed with water, soda and finally with water until neutral. After drying, the ether is evaporated and the residue recrystallized from acetone or ligroin.

Table II gives the physical constants of these compounds as well as the substances isolated from

the solutions on which the rotations were determined. Table III summarizes the analytical results.

TABLE II

	Enol ethyl ethers	Cholestenone	Testosterone propionate	Testosterone benzoate
M. p., °C.	Freshly prepd. material	83.5–85, clear 95	143–150	Soften 175, 181–192
	Mixed with free ketone	69–70	103–133	Soften 130, 156–168
	Recl. $CHCl_3$ from rotn.	79–81	...	185–189
	Mixed with free ketone	80–81	...	186–190
Rotation	Initial	−102.3	−140	− 42.5
	Final	+ 87.7	+ 88	+143
	Free initial ketone	+ 87	+ 88	+148

TABLE III

FORMULAS AND ANALYSES

Enol ethyl ethers		Cholesterone	Testosterone propionate	Testosterone benzoate
Formulas		$C_{29}H_{48}O$	$C_{24}H_{36}O_3$	$C_{28}H_{36}O_3$
Carbon, %	Calcd.	84.46	77.42	80.00
	Found	84.2	76.89	...
Hydrogen, %	Calcd.	11.65	9.67	8.57
	Found	11.53	10.12	...
C_2H_5O, %	Calcd.	10.92	12.09	10.71
	Found	9.77	...	10.25

SCHERING CORPORATION
BLOOMFIELD, N. J.

E. SCHWENK
G. FLEISCHER
B. WHITMAN

RECEIVED JUNE 15, 1938

THE QUANTITATIVE DETERMINATION OF AMINO ACIDS

Sir:

One of the methods frequently employed for the estimation of individual amino acids in protein hydrolysates involves the selective precipitation of the respective amino acids in the form of salts. It is the object of such methods to precipitate as much of the salt as possible, 100% isolation being the ultimate goal. Unfortunately, most of the salts that are precipitated selectively are not precipitated quantitatively. Such an incomplete precipitation may be used to advantage, however, in a newly developed analytical principle which permits a rather precise determination of individual amino acids. It depends upon the fact that the amount of an amino acid salt precipitated at equilibrium is a function of the concentration of its ions in solution.

In order to demonstrate this, let us assume that an acidic reagent, RH, forms, with an amino acid, A, the binary salt, AH·R. In many cases the so-called solubility product of the participating ions was found to be approximately a constant.

$$[AH^+] \times [R^-] = \text{const.} \qquad (1)$$

Figure 1 illustrates an experiment in which increasing amounts r', r'', r''' of the reagent are added to several equal portions of a solution, each containing Y moles of amino acid. In each sample a precipitate is formed. Were it completely insoluble, the course of precipitation would follow the straight line OB. In reality, however, the precipitation follows a hyperbolic curve passing through the points C', C'', C''', C''''.

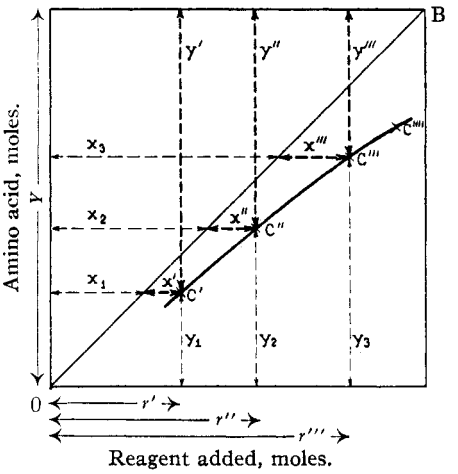


Fig. 1.—Determination of amino acids by means of the solubility product.

The precipitate obtained with an amount of reagent r' contains equivalent amounts x_1 of the reagent, and y_1 of the amino acid; the amounts x' of the reagent and y' of the amino acid remain in solution. Similarly, in a precipitation with an amount of reagent r'' the amounts x'' and y'' remain in solution. From equation (1) it follows that

$$x' \times y' = x'' \times y'' = x''' \times y''' \qquad (2)$$

Experimentally we have found the requirements of equation (2) to be fulfilled satisfactorily for the precipitation of proline by rhodanilic acid [M. Bergmann, *J. Biol. Chem.*, **110,** 471 (1935)], of glycine, alanine and leucine by dioxypyridic acid [M. Bergmann, *ibid.*, **122,** 569 (1938)], and of tyrosine by dioxanilic acid. In all cases where equation (2) is valid, the amount Y of an amino acid can be determined in a solution of unknown content. For this purpose equation (2) may be written in the following manner:

$$x'(Y - y_1) = x''(Y - y_2) = x'''(Y - y_3) \qquad (3)$$

In equation (3) all values, with the exception of Y, may be determined experimentally.

In order to demonstrate the precision of such determinations, we report the following analyses: