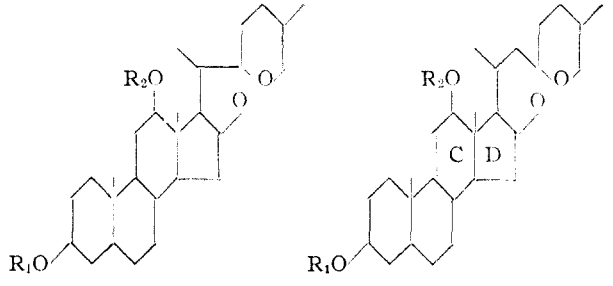
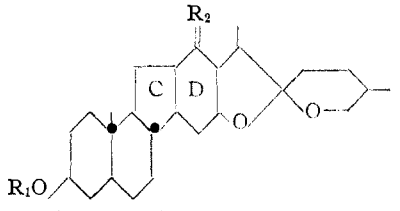
figurations at C_{12} for I and II. Confirmation of this configurational assignment was adduced by the markedly more rapid consumption of two equivalents of alkali by IIa in contrast to Ia. This is again in accord with the observed saponification rates for the corresponding C_{12}-epimeric acetoxy cholanates,[1a] and in conformity with the greater ease of hydrolysis associated with equatorial ester groupings.[2] Rockogenin[3] isolated from natural sources was found to be identical with II and should, therefore, be assigned the 12(β)-OH configuration.



(I) $R_1 = R_2 = H$ (II)
(Ia) $R_1 = R_2 = CH_3CO^-$ (IIa)
(Ib) $R_1 = CH_3O_2C(CH_2)_2CO^-$ (IIb)
$R_2 = CH_3SO_2^-$



(III) $R_1 = CH_3CO$; $R_2 = CH_2$
(IV) $R_1 = H$; $R_2 = O$

I and II were converted by successive succinolylation and treatment with methanesulfonyl chloride in pyridine to: Ib m.p. 185–188° (dec.), $[\alpha]^{25}D$ −13.4° (chf.). Found: C, 63.96; H, 8.50; S, 5.28, and IIb, m.p. 127–132° (dec.), $[\alpha]^{24}D$ −48.4° (chf.). Found: C, 63.85; H, 8.45; S, 5.18, respectively. Solvolysis of IIb in refluxing methanol or *t*-butanol with or without added alkali alkoxide, produced an olefin isolated as its acetate (III) m.p. 221–225°, $[\alpha]^{23}D$ −80.6° (chf.). Found: C, 76.21; H, 9.92, with bands in the infrared at 6.04 and 11.24 μ. Under the same conditions of solvolysis Ib was recovered essentially unchanged.

Hydroxylation of III with osmium tetroxide followed by saponification yielded a triol m.p., *ca.* 200°, converted quantitatively to a diacetate m.p. 212–216°. Found: C, 70.30; H, 9.37, having an OH band in the infrared at 2.8 μ. Saponification of this diacetate quantitatively regenerated the triol, and the latter on cleavage with periodic acid produced 60–65% of formaldehyde, determined by chromotropic acid titration[4] and isolation of its dimedone derivative m.p. and m.m.p. with an authentic sample 191°, together with a quantitative yield of a *nor*-ketone (IV) m.p. 180–183°; $[\alpha]^{24}D$ −93.8° (chf.). Found: C, 75.01; H, 9.66, having a single carbonyl band in the infrared at 5.84 μ and only end absorption in the ultraviolet.

The formation of III from rockogenin by C/D ring contraction and expansion represents a rearrangement path wherein the stereoelectronic requirements are fulfilled only in the case of the natural C_{12}-β-configuration II.[5] The significance of this geometrical factor is reflected in the extraordinary ease with which this rearrangement occurs.[6] This C/D ring structural type has been advanced recently in the formulation of jervine[7] and veratramine.[8] The present work constitutes the first chemical realization of this abnormal steroid system and affords thereby not only support for the C/D ring structures assigned to these alkaloids but also suggests a highly probable mode in their biogenesis.

(2) D. H. R. Barton, *Experientia*, **6**, 316 (1950).

(3) Kindly supplied by Dr. L. A. Sweet of Parke, Davis & Co., Detroit, Michigan.

(4) C. E. Bricker and W. A. Vail, *Ind. Eng. Chem., Anal. Ed.*, **22**, 720 (1950).

(5) Compare the rearrangement-dehydration of β- and *epi*-β-amyrin; *cf.* ref. 2. Also O. Jeger "Fortschritte der chemie organischer Naturstoffe," 1950, Vol. VII, p. 65.

(6) Compare, for example, S. Winstein, B. K. Morse, E. Grunwald, H. W. Jones, J. Corse, D. Trifan and H. Marshall, THIS JOURNAL, **74**, 1127 (1952).

(7) J. Fried, O. Wintersteiner, M. Moore, B. M. Iselin and A. Klingsberg, *ibid.*, **73**, 2970 (1951).

(8) Ch. Tamm and O. Wintersteiner, Abstr. of the Meeting-in-Miniature of the Am. Chem. Soc., Newark, N. J., Jan. 28, 1952.

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C. STEWARD SNODDY, JR.
N. L. WENDLER

RECEIVED MARCH 31, 1952

THE AMMONIA INDUCED DECOMPOSITION OF NITRIC OXIDE

Sir:

This is to report a serious flaw in the experimental technique of a recent investigation of the same title,[1] which invalidates its major conclusions. In that work, the extent of the decomposition of NO was determined by measuring the pressure of nitrogen in a previously evacuated bulb, partly immersed in liquid nitrogen and connected to the reaction vessel by capillary tubing. This procedure leads to entirely erroneous nitrogen pressures since nitrogen is enriched far above its correct partial pressure in the bulb when it is opened to the reaction vessel. Mass flow will bring the gas mixture instantaneously from the large reaction vessel into the bulb where all condensable gases freeze out and leave a nitrogen pressure much lower than that of the gas mixture remaining in the quartz vessel. The process therefore continues until the pressure of nitrogen in the bulb is equal to that of the gas mixture in the reaction vessel, still of its original composition, and therefore containing much nitric oxide. From then on, it is diffusion of NO into the bulb and diffusion of N_2 back into the reaction vessel which would take the system to its final state, but diffusion through capillary tubing is an exceedingly slow process.

We tested the correctness of this objection in various ways: A. By preparing known mixtures of NO and N_2, following the procedure used in the reported note and obtaining measured "N_2 pressures" which were always much too high. B. By repeating some actual NO–NH_3 experiments

(1) C. P. Fenimore and J. R. Kelso, THIS JOURNAL, **74**, 1593 (1952).

under exactly the same conditions as those given by Fenimore and Kelso, measuring N_2 in the manner reported and then allowing the gas mixture which remained in the reaction vessel to by-pass the cold bulb and expand into an evacuated flask. To this gas excess oxygen was added and immediately a dark yellow color developed, indicating much unreacted nitric oxide. C. Finally, by repeating a number of NO–NH_3 runs using a method of measuring N_2 pressures which is not open to the same objections.

In this method, an evacuated bulb at room temperature is opened to the reaction vessel at the prescribed time. The bulb is then isolated from the reaction vessel and the nitrogen pressure measured after cooling the bulb in liquid nitrogen in a reproducible manner. Pressure corrections are then applied to allow for the expansion of the reaction mixture into the bulb and also for the cooling of the bulb when pressures are read.

Table I presents a summary of results in comparison with the recently reported data.

TABLE I

	Reactants, mm.		Heating time,	P_{N_2}, mm.	
T, °C.	NO	NH_3	min.	This work	F. & K.[1]
700	465	18.5	60	20.5	120
740	697	6	5	6	35
740	700	6	15	8	60
740	704	6	30	8	70
755	688	16.5	10	26	130

It is apparent from the data that there is only little nitrogen made. Thus, there is no need nor justification to assume an induced or catalyzed decomposition of nitric oxide, since the observed nitrogen pressures are generally consistent[2] with the simple stoichiometric reaction

$$2NH_3 + 3NO \longrightarrow 2^1/_2N_2 + 3H_2O$$

Further work on the kinetics and mechanism of this reaction are in progress here.

(2) Because of the large pressure correction factors, the probable error of the reported nitrogen pressures is rather high.

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RECEIVED MAY 2, 1952

A NEW METHOD FOR ADDING AMINO ACIDS AND PEPTIDES TO PROTEINS

Sir:

The reaction of N-carboxyamino acid anhydrides in water is mainly one of hydrolysis to the amino acids and carbon dioxide.[1] We have found, however, that the anhydrides will polymerize to form high yields of polypeptides if the aqueous solutions are buffered at *p*H's near neutrality, and that the rate of polymerization is increased by the use of amine initiators.[2] These findings suggested that proteins might also be used to initiate the polymerization, thus allowing the addition of amino acids and peptides to the protein under very mild conditions. Accordingly, crystalline bovine plasma albumin and crystalline chymotrypsin were treated with N-carboxyglycine anhydride in phosphate buffered solutions at *p*H 7.4. The reaction mixtures were initially cooled to 4° and then allowed to warm to room temperature. After several hours, the insoluble polyglycine was separated by centrifugation, and the clear solution dialyzed for 72 hours to remove all glycine and soluble glycine peptides. Aliquots were then hydrolyzed and analyzed for glycine by chromatography on Dowex-50,[3] by microbiological assay using *Leuconostoc mesenteroides*,[4,5] and by a colorimetric chemical method.[6] Control experiments in which the anhydride was replaced by glycine or polyglycine were run. The results of experiments with two proteins are shown in Table I. These data show that the glycine content of the plasma albumin was increased ten-fold, and that of chymotrypsin, about four-fold. The yields were quantitative based on the protein, and about 25% based on the anhydride. The molecular weights were increased by about 12% with both proteins. In spite of the large increase in glycine content, both proteins remained completely soluble with no evidence of denaturation, and the chymotrypsin showed no loss in enzymatic activity.[5] In preliminary electrophoretic experiments the polyglycyl-albumin showed a single peak. It was readily precipitated by antiserum prepared against normal plasma albumin.[5]

TABLE I

REACTION OF N-CARBOXYGLYCINE ANHYDRIDE WITH BOVINE PLASMA ALBUMIN AND CHYMOTRYPSIN

Reactants[a]	Glycine/ 100 g. protein, g.	Molecular weight	Moles glycine/ mole protein	Moles glycine added/ mole protein
Albumin alone	1.9	69,000	18	...
Albumin + anhydride	18.3	77,600[b]	189	171
Albumin + glycine	2.0	69,000	18	0
Chymotrypsin alone	6.8	27,000	25	...
Chymotrypsin + anhydride	22.3	30,200[b]	90	65
Chymotrypsin + polyglycine	6.7	27,000	25	0

[a] Reaction mixture contained 800 mg. of anhydride, glycine or polyglycine per 100 ml. of 1% protein in *M*/15 phosphate buffer at *p*H 7.4. [b] Calculated from glycine added.

From the known chemical properties of the N-carboxyamino acid anhydrides, it would be expected that reaction would most likely occur with amino groups of the protein. In addition, other groups might react. The reaction may add one or more amino acid residues per reaction site. A *p*H titration of the polyglycylalbumin revealed a marked shift in the *p*H region 7 to 11. From the extent of this shift, it is estimated that the anhydride reacted with about a third of the amino groups. This requires that, on an average, the glycine was attached as polypeptides.

Thus, we have for the first time attached unsubstituted amino acids and peptides to native

(1) E. Katchalski, *Adv. Prot. Chem.*, **6**, 140 (1951).

(2) R. R. Becker and M. A. Stahmann, Am. Chem. Soc., Milwaukee Meeting, April, 1952, p. 35C.

(3) W. H. Stein and S. Moore, *Cold Spring Harbor Symposia Quant. Biol.*, **14**, 179 (1950).

(4) L. M. Henderson and E. E. Snell, *J. Biol. Chem.*, **172**, 15 (1948).

(5) We wish to thank Mr. J. C. Alexander for the microbiological assays, Mr. J. E. Casida for the chymotrypsin assays, and Mr. T. Makinodan for the immunological tests.

(6) R. Krueger, *Helv. Chim. Acta*, **32**, 238 (1949).