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).

proteins, forming the peptide bonds under such mild conditions that denaturation does not occur. We have utilized this method with other proteins and other amino acids with essentially similar results. It would seem to be applicable to many amino acid anhydrides and a wide variety of proteins. These studies, as well as an investigation of the relative reactivity of various groups of the protein which may be acylated by the anhydrides, are under investigation. The effects upon the biological activity of enzyme and virus proteins are also being studied.

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RECEIVED MARCH 17, 1952

SYNTHESIS OF 11-KETO STEROIDS

Sir:

In an earlier communication[1] we reported a synthesis of *allo*pregnan-3β-ol-11,20-dione acetate (I) from ergosterol, stigmasterol, cholesterol and diosgenin. In this communication we wish to present a second, more direct, method for the preparation of the *allo* diketo pregnane (I) from these same raw materials. Heusser and his co-workers[3] have reported the isomerization of the mono-epoxide derived from $\Delta^{7,9(11),22}$-ergostatrien-3-β-ol acetate[2] by the action of boron trifluoride etherate in benzene solution at room temperature to $\Delta^{8,22}$-ergostadien-3β-ol-11-one acetate (II) and we have independently carried out the same reaction in 80% yield; m.p. 131.5–134°; $[\alpha]_D$ +110° ($CHCl_3$); λ_{max} 254 mμ, E_M 9140 (alc.); found: C, 79.19; H, 10.31. Saponification of II yielded $\Delta^{8,22}$-ergostadien-3β-ol-11-one (III); m.p. 171–173°; $[\alpha]_D$ +135° ($CHCl_3$); λ_{max} 254 mμ, E_M 8920 (alc.); found: C, 81.54; H, 10.45.

As expected, both II and III are inert to reaction with 2,4-dinitrophenylhydrazine. Irrefutable structure proof of these ketones as well as a new and shorter path to I was provided by the reduction of the Δ^8-11-ketone (II) with lithium and liquid ammonia in 85–90% yield to Δ^{22}-ergostene-3β-ol-11-one (IV), m.p. 168–169.5°; $[\alpha]_D$ +23° ($CHCl_3$). The identity of the lithium-ammonia reduction product of II was conclusively demonstrated by comparison with the product previously obtained by the Wolff–Kishner reduction of Δ^{22}-ergostene-3β-ol-7,11-dione acetate.[1]

Similarly diosgenin acetate was converted via the monoepoxide to Δ^8-spirostene-3-β-ol-11-one acetate (8-dehydro-11-keto tigogenin acetate); m.p. 235–238°; $[\alpha]_D$ +57° ($CHCl_3$); λ_{max} 255 mμ; E_M 9000 (alc.); found: C, 73.62; H, 8.89; which was likewise reduced by lithium–liquid ammonia to the previously described spirostan-3β-ol-11-one.[1]

(1) E. M. Chamberlin, W. V. Ruyle, A. E. Erickson, J. M. Chemerda, L. M. Aliminosa, R. L. Erickson, G. E. Sita and M. Tishler, THIS JOURNAL, **73**, 2396 (1951).

(2) In accordance with the Swiss investigators,[3] we believe the monoepoxides prepared from allo-$\Delta^{7,9(11)}$-steroids are Δ^{7}-9α,11α-epoxides. However, R. C. Anderson, R. Budziarek, G. T. Newbold, R. Stevenson and F. S. Spring regard the epoxide above to be a $\Delta^{9(11)}$-7,8-epoxide; *Chem. and Ind.*, 1035 (1950). Further discussion of this problem will be forthcoming in a later communication.

(3) H. Heusser, K. Eichenberger, P. Kurath, H. R. Dallenbach and O. Jeger, *Helv. Chim. Acta*, **34**, 2106 (1951).

Of interest to the structure problem of these monoepoxides, as well as their utilization for the synthesis of I, is the fact that acids in aqueous or polar media effect rearrangement to 7-ketones rather than 11-ketones. Thus the mono-epoxide from $\Delta^{7,9(11),22}$-ergostatrien-3β-ol; m.p. 188–189°; $[\alpha]_D$ −34° ($CHCl_3$); found: C, 81.25; H, 10.79; (obtained either by the direct action of perbenzoic acid or by alkaline hydrolysis of the 3-acetate) is converted by the action of 0.07 *N* sulfuric acid in aqueous–acetone at 25–30° for ten to thirty minutes to $\Delta^{9(11),22}$-ergostadien-3β-ol-7-one (V); m.p. 153.5–154.5°; $[\alpha]_D$ −53° ($CHCl_3$); found: C, 82.10; H, 10.40; end absorption above 220 mμ; 3-acetate: m.p. 176–177°; $[\alpha]_D$ −43.5° ($CHCl_3$); found: C, 78.89; H, 9.91.[4] By the action of dilute sulfuric acid on the above mono-epoxide at 50–70° for a few hours, or by treatment of V with alcoholic alkali at room temperature, $\Delta^{8,22}$-ergostadiene-3β-ol-7-one (VI) is obtained; m.p. 179–180°; $[\alpha]_D$ −43° ($CHCl_3$); λ_{max} 253 mμ, E_M 9970 (alc.); found: C, 82.11 H, 10.98. Acetylation of VI yielded the 3-acetoxy derivative, m.p. 213–213.5°; $[\alpha]_D$ −59° ($CHCl_3$); which had been obtained previously in low yield by Stavely and Bollenback[5] from the oxidation of $\Delta^{7,22}$-ergostadien-3β-ol acetate by chromic acid. Both V and VI yielded 2,4-dinitrophenylhydrazones.

Complete details of this work will be published later in THIS JOURNAL.

(4) Under similar conditions, 0.3 *N* sulfuric acid in aqueous dioxane for three minutes, O. Jeger and his co-workers[3] obtained $\Delta^{8,22}$-ergostadien-3β,7(?),11α-triol-3-monoacetate.[1] The sensitivity of the triol to acids suggests that the time allowed for reaction is probably the most critical factor in determining the course of action. The β,γ-unsaturated ketone has also been obtained by the action of dilute acids on the triol.

(5) H. E. Stavely and G. N. Bollenback, THIS JOURNAL, **65**, 1285 (1943).

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STEROIDAL SAPOGENINS. XXIII.[1] INTRODUCTION OF THE 11-KETO AND 11α-HYDROXY GROUPS INTO RING C UNSUBSTITUTED STEROIDS (PART 6). NEW APPROACH TO 11-OXYGENATED STEROIDS

Sir:

Heusser and co-workers[2] reported recently that mono-epoxides of certain steroidal $\Delta^{7,9(11)}$-dienes upon treatment with boron trifluoride yield the corresponding Δ^8-11-ketones which would constitute a proof for the Δ^7-9,11-oxido structure of the starting mono-epoxides. The structure assignment of the unsaturated ketone rested essentially on the non-reactivity of the carbonyl group and the substance's non-identity with the Δ^8-7-ketone. We should now like to report certain experiments in the sapogenin series which not only prove un-

(1) Paper XXII, C. Djerassi, E. Batres, J. Romo and G. Rosenkranz, THIS JOURNAL, **74**, June (1952).

(2) H. Heusser, *et al.*, *Helv. Chim. Acta*, **34**, 2106 (1951); **35**, 295 (1952).