Modification of the structure of X by the introduction of larger alkyl groups as in the 1,4′-bis-ethiodide (XI), the 1,4′-bis-butiodide (XIII) and the tetraethyl salt (XVI) gave compounds of essentially the same potency as antagonists of I and (C). The antagonistic activity of the more heavily alkylated derivatives was accompanied in some cases by a slight additive curariform activity, thus making these members less desirable for use than X.

A detailed pharmacological report will be published elsewhere.

Acknowledgment.—The author is indebted to S. W. Blackman for the microanalytical results reported, and to Drs. E. J. de Beer and C. H. Ellis, and to J. C. Castillo, R. V. Fanelli and A. L. Wnuck who kindly supplied the pharmacological data summarized here.

Experimental

These compounds were prepared in simple modifications of the procedures described previously.[1] Details for all the bis-quaternary ammonium salts are summarized in Table I. Preparations for new intermediates and for compound VI are given below.

4-(4′-Dimethylamino)-stilbazole Ethiodide.—This was prepared by the condensation[3] of 4-methylpyridine ethiodide and 4-dimethylaminobenzaldehyde. Bright red crystals were obtained by recrystallization from methanol; yield 100%; m.p. 255–260° (dec.).

Anal. Calcd. for $C_{17}H_{21}N_2I$: C, 53.7; H, 5.6. Found: C, 54.0; H, 5.6.

1-Ethyl-4-(4′-dimethylamino)-stilbazoline Hydriodide.—Catalytic hydrogenation[4] of the 4-(4′-dimethylamino)-stilbazole ethiodide gave the stilbazoline hydriodide as white crystals from methanol–ether mixtures; yield 90%; m.p. 127–128°.

Anal. Calcd. for $C_{17}H_{29}N_2I$: C, 52.6; H, 7.5; I, 32.8. Found: C, 52.5; H, 7.3; I, 32.9.

2-(4′-Dimethylamino)-stilbazole Ethiodide.—This compound was obtained by the condensation[3] of 2-methylpyridine ethiodide with 4-dimethylaminobenzaldehyde and after recrystallization from methanol gave 100% of bright red crystals; m.p. 258–259°.

Anal. Calcd. for $C_{17}H_{21}N_2I$: C, 53.7; H, 5.6. Found: C, 53.6; H, 5.6.

1-Ethyl-2-(4′-dimethylamino)-stilbazoline Hydriodide.—Catalytic hydrogenation[4] of the 2-(4′-dimethylamino)-stilbazole ethiodide gave the stilbazoline hydriodide; white crystals from methanol–ether; m.p. 145–146°; yield 97%.

Anal. Calcd. for $C_{17}H_{29}N_2I$: C, 52.6; H, 7.5. Found: C, 52.7; H, 7.6.

1-Methyl-4-(4′-methylethylamino)-stilbazoline 1,4′-Bismethiodide (VI).—A mixture of 1.3 g. (0.0035 mole) of 1-methyl-4-(4′-dimethylamino)-stilbazoline hydriodide[4] and 5 cc. (large excess) of ethyl iodide was refluxed on a steam-bath for four hours. Excess ethyl iodide was evaporated and the residue was washed repeatedly with ether by decantation. The viscous residue was stirred up with 10 cc. of saturated aqueous potassium carbonate solution and the insoluble crystalline precipitate was filtered off by suction, washed with saturated aqueous potassium carbonate, and finally washed once with a little cold water. The product, 1.6 g. (90%), was 1-methyl-4-(4′-dimethylamino)-stilbazoline 4′-monoethiodide, and this when refluxed in methanol with excess methyl iodide gave 80% of VI as white crystals from methanol–ethyl acetate; m.p. 185–186°.

TUCKAHOE 7, NEW YORK

[CONTRIBUTION FROM THE CHEMICAL RESEARCH DIVISION, SCHERING CORPORATION]

Catalytic Isomerization of Spirostans to Furostenols[1]

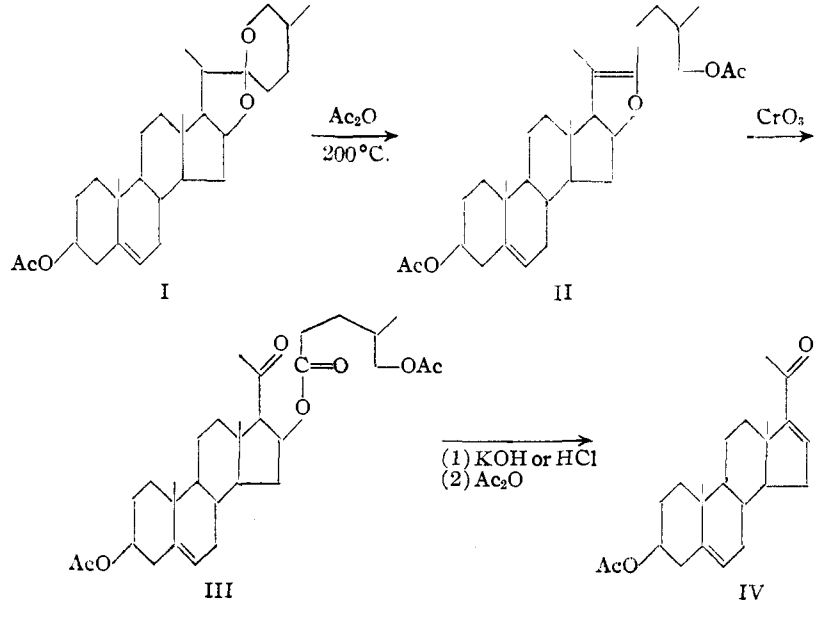
BY DAVID H. GOULD, HEINZ STAEUDLE AND E. B. HERSHBERG

RECEIVED AUGUST 3, 1951

Catalysts of the Lewis acid type have been found which permit the isomerization of spirostans to furostenols in acetic anhydride solution at the boiling point rather than at 200°.

In order to convert diosgenin acetate (I, 22-iso-5-spirostan-3β-ol acetate) into 5,16-pregnadien-3β-ol-20-one acetate (IV) according to scheme (1),[2] it must first be isomerized to the pseudo compound, 5,20(22)-furostadiene-3β,26-diol diacetate (II). The conditions of this reaction reported by Marker and Rohrmann[2] involved the use of acetic anhydride at elevated temperature and

(1)



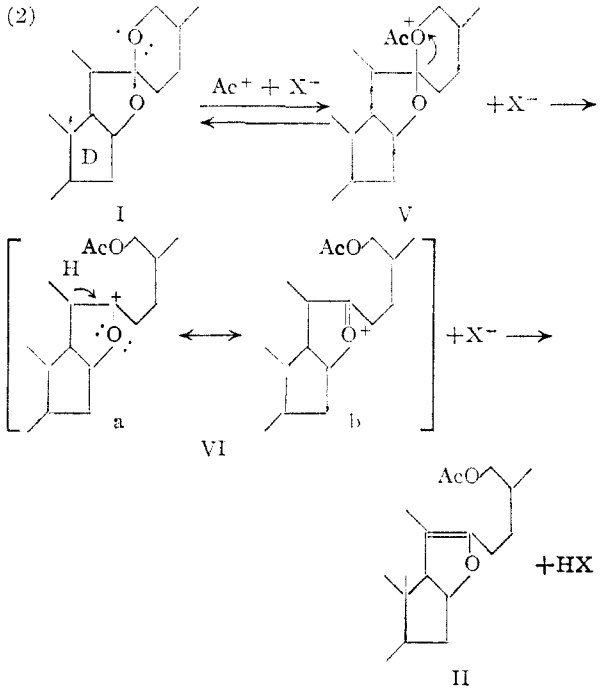
(1) Presented at the XIIth International Congress of Pure and Applied Chemistry, New York City, September 11, 1951.

(2) R. E. Marker, R. B. Wagner, P. R. Ulshafer, E. L. Wittbecker, D. P. J. Goldsmith and C. H. Ruof, THIS JOURNAL, **69**, 2167 (1947); R. E. Marker and E. Rohrmann, *ibid.*, **61**, 3592 (1939).

pressure, while the obvious use of higher boiling anhydrides as solvents led to lower yields.[3] Examination of a possible mechanism of the reaction suggested various reagents which might facilitate the reaction.

It seemed likely that the mechanism of the isomerization involved the acetylonium ion, formed at high temperature, as shown in scheme (2), X = AcO. This ion, a strong Lewis acid, would

(2)



reversibly attack the nucleophilic pyran oxygen. The complex V could readily open to give the resonance stabilized hybrid, VI a,b. Finally the favored elimination of the proton at C_{20} would yield a stable molecule irreversibly (II, 5,20(22)-furostadiene-3β,26-diol diacetate).[4]

On the basis of this analysis, it should be possible to catalyze the reaction with many strong Lewis acids, and this proved to be true. When 22-iso-5-spirosten-3β-ol acetate (I) was heated for several hours in acetic anhydride solution on the steam-bath or at the boiling point, and treated with hydrochloric acid, acetyl chloride, aluminum chloride, or *p*-toluenesulfonic acid, it was possible to isolate 5,20(22)-furostadiene-3β,26-diol diacetate (II), m.p. 98–100°. The reaction could be followed by the decrease in the saponification equivalent, or by the chromatographic separation of the product and starting material. The product did not differ in infrared spectrum from material made by the autoclave process.[5] It was saponified to the free furostadienediol (pseudodiosgenin), which was treated with acid to obtain the original iso-spirostenol (diosgenin) thus showing no deep seated rearrangement.[6] As further proof, the material was oxidized, hydrolyzed and acetylated by known procedures to give 5,16-pregnadien-3β-ol-20-one acetate (IV).[2,6] Table I shows some of the conditions and results.[7]

TABLE I[a]

PREPARATION OF 5,16-PREGNADIEN-3β-OL-20-ONE ACETATE

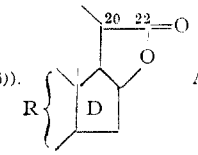
Expt.	Catalyst	Amount, g.	Reflux time, hr.	Product wgt., g.	M.p., °C.	$E^{1\%}_{1cm.}$ at 233 mμ	Purity, %
1	Conc. HCl	14.9 ml.	2	5.2	165–170	264	96.0
2	*p*-TsOH	2	8	0.9	170–173	275	100
3	*p*-TsOH	1	16[b]	Mostly unchanged diosgenin acetate			
4	AcCl	8.55	4	4.4	165–171	259	94.3
5	$AlCl_3$	5	3	13.8	163–170	260	94.5
6	$AlCl_3$	5	24[c]	7.87	162–170	266	96.7
7	$AlCl_3$	10	3	8.7	165–171	253	92.1
8	$AlCl_3$	0.5	8[b]	Mostly unchanged diosgenin acetate			
9	$AlCl_3$	2	4	5.9	170–173	275	100

[a] Fifty g. of diosgenin acetate refluxed in 100 ml. of Ac_2O. [b] Mixture not oxidized, since diosgenin acetate crystallized out. [c] Heated on steam-bath with stirring.

When the isomerization product (II) was not isolated, the reaction mixture was treated with sodium acetate, after which a selective oxidation with chromic acid could be achieved, with attack occurring at the 20,22-bond only.[6,8,9] The oxidation gave 5-pregnene-3β,16-diol-20-one-3-acetate-16-(δ-acetoxy-γ-methyl valerate) (diosone diacetate) (III).[6] Under the acid conditions of the reaction, this material partially loses the ester side chain to produce pregnadienolone (IV). The crude material in one case (see Experimental) had the ultraviolet absorption spectrum characteristic of IV to the extent of 33%. The cleavage is usually completed by saponification with alcoholic base or mineral acid.[6] The saponification with alcoholic base causes some loss of Δ^{16}-20-ketosteroid, since the unsaturated ketone is partially converted to the

(3) R. E. Marker and E. Rohrmann, THIS JOURNAL, **62**, 518 (1940).

(4) There is evidence that a double bond may form between C_{22} and C_{23} under some circumstances. Oxidation of spirostans gives small amounts of C_{22} lactones (see R. Tschesche and A. Hagedorn, *Ber.*, **68**, 1412 (1935)).

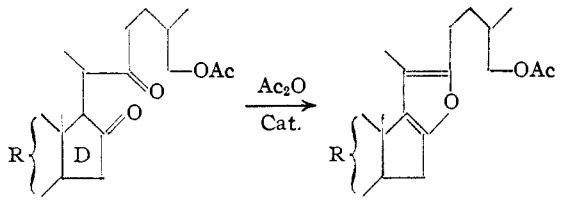


Also, bromination of spirostans gives 23-bromo derivatives (R. E. Marker, D. L. Turner, A. C. Shabica and P. R. Ulshafer, THIS JOURNAL, **63**, 1032 (1941)).

(5) R. E. Marker, T. Tsukamoto and D. L. Turner, *ibid.*, **62**, 2525 (1940).

(6) R. E. Marker, D. L. Turner, R. B. Wagner, P. R. Ulshafer, H. M. Crooks, Jr., and E. L. Wittle, *ibid.*, **63**, 774 (1941).

(7) After this work was completed, U. S. Patent 2,535,073 was issued to St. Kaufmann and G. Rosenkranz, which involved the use of *p*-toluenesulfonic acid to catalyze the formation of furostadiene diacetates from sapogenin diacetates of the kryptogenin type, *e.g.*



They state, however, that the catalysis is useful only in this special case, whereas we have shown a more general applicability and have outlined the group of catalysts which facilitate this isomerization.

(8) R. E. Marker, THIS JOURNAL, **62**, 3350 (1950).

(9) When no sodium acetate was added, oxidation was not selective and gave a product (not isolated) which absorbed ultraviolet light at 275 mμ, possibly an α-diketone. There was no ultraviolet absorption in this region before oxidation.

16-alkoxy-20-ketosteroid[10]; therefore acid hydrolysis is preferable. Alkaline hydrolysis in a two-phase system could also be used to advantage. Furthermore, cleavage was achieved by heat alone, since treatment of the crude diosone diacetate mixture with boiling xylene increased the ultraviolet absorption about 50% from E = 91 to E = 137 at 235 mμ. This crude product was then crystallized from acetic anhydride and gave pregnadienolone acetate of good purity.

Discussion

The catalyst must function essentially according to (3), to supply acetylonium ions.

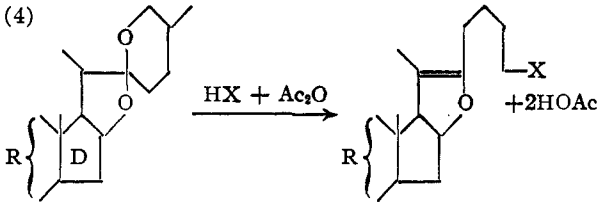
(3) (a) $Ac_2O + HX \rightleftharpoons AcX + HOAc$

(b) $AcX \underset{\Delta}{\rightleftharpoons} Ac^{\oplus} + X^{\ominus}$

The Ac–X bond is sufficiently weaker than the Ac–OAc bond to permit (3b) to occur at moderate temperatures. In the case of acetyl chloride, AcX is already present. Aluminum chloride is reported[11] to react with acetic anhydride to give acetyl chloride and an aluminum complex, and is therefore not truly a catalyst. HX (X = Cl), however, is regenerated as in (2) and functions as the catalyst.

It is evident from Table I (3 and 8) that the acid must exceed a minimum value, since at lower concentrations the isomerization is not complete even after 16 hours. It seems likely that the acid may be partially utilized for ester formation as in (4)

(4)



On the other hand greatly increasing the amount of aluminum chloride (Table I, 7) leads also to lowered yields, and some other strong acids gave no recognizable products. This is presumably due to further attack on the steroid, possibly at the furan ring.

Experimental[12]

5,20(22)-Furostadiene-3β,26-diol Diacetate (II).—Twenty grams (0.0436 mole) of 22-iso-5-spirosten-3β-ol acetate (I) was dissolved in 100 ml. of acetic anhydride and treated with 5.8 g. (0.0436 mole) of aluminum chloride. The mixture was refluxed for four hours, cooled and then poured into ice and water. The precipitated tan resin slowly solidified. The crude material (18.6 g.) was dissolved in ether and the solution was treated with Darco and filtered. Concentration and addition of methanol gave crystals which were collected and washed with methanol–acetone mixture. The product weighed 17.0 g., m.p. 93–98°.

A sample of this crude material was sublimed and chromatographed on Florisil. The fraction eluted with benzene was crystallized from ether–methanol to give a product of m.p. 98.5–100.5° (lit.[5] 100–101°). There was no depression of melting point with an authentic sample of II, m.p. 93–97°, prepared by the isomerization of isospirostenol acetate in acetic anhydride solution at 200° for eight hours,[5] and the infrared spectra did not differ. The rotation was $[\alpha]^{25}D$ −47.0°. The saponification equivalent of the crude product was 227, 225; calcd. for furostadienediol diacetate, 249. The corresponding value of the starting material was 461; calcd. for isospirostenol acetate, 456.

Preparation of 22-Iso-5-spirosten-3β-ol from II.—A sample of 5 g. of II prepared by the catalytic procedure, m.p. 95–98°, was treated with 2 g. of potassium hydroxide in 20 ml. of ethanol and refluxed for one hour. The mixture was poured into water, extracted with ether and the ether solution dried and evaporated. The residue (4.7 g.) was crystallized from acetone–ethanol to give 2.4 g. of crude 5,20(22)-furostadiene-3β,26-diol, m.p. 165–171° (lit.[5] 172–174° or 190–204°). When mixed with isospirostenol, the product melted at 147–181°.

A sample of 0.9 g. was dissolved in 100 ml. of anhydrous ethanol and treated with 5 ml. of concd. hydrochloric acid. The solution was refluxed for two hours and allowed to stand 18 hours. The mixture (containing crystals) was concentrated to 40 ml., chilled and filtered. The product was crude isospirostenol, m.p. 193–201°.

Two recrystallizations from ethyl acetate gave 0.62 g. of material, m.p. 206–208°, which showed no depression of melting point when mixed with an authentic sample of m.p. 208–210°.

Separation of I and II.—One gram each of crude I (m.p. 170–185°, $[\alpha]D$ −113°) and crude II (m.p. 90–95°) were dissolved in a mixture of 5 ml. of benzene and 40 ml. of hexane. The solution was adsorbed on a column of Florisil (100–200 mesh) and the column was eluted with 250 ml. of hexane. The residue after evaporation was 0.995 g. of crude I, m.p. 180–190°, $[\alpha]D$ −115°. Recrystallizations from ethyl acetate and isopropyl alcohol gave 0.75 g. of isospirostenol, m.p. 196–199° (lit.[4] 199–200°), $[\alpha]D$ −119.0°.

Elution with 300 ml. of 10% benzene in hexane gave 0.205 g. of II, m.p. 95–98°, $[\alpha]^{23}D$ −48°, and elution with 300 ml. of benzene gave 0.44 g., m.p. 96–98°, $[\alpha]^{24}D$ −45°. The oily remainder was eluted with 2% methanol in benzene.

5,16-Pregnadien-3β-ol-20-one Acetate (IV). (a) Use of Aluminum Chloride.—A sample of 50 g. of I was suspended in 100 ml. of acetic anhydride and treated with 5 g. of aluminum chloride. The mixture was refluxed for three hours, cooled and treated with 12.5 g. of sodium acetate.

The mixture containing II was diluted to 750 ml. with acetic acid, stirred at 11–13° and treated dropwise with a solution of 18 g. of chromic anhydride in 25 ml. of water and 50 ml. of acetic acid, added over 15 minutes. After the mixture was stirred 20 minutes more, the excess chromic acid was reduced with a solution of 6 g. of sodium bisulfite in water. The mixture was concentrated *in vacuo* to 200 ml. and diluted with 200 ml. of salt water. It was then extracted twice with 350 ml. of benzene and the combined benzene layers were washed neutral with salt water, sodium bicarbonate solution and salt water.

The benzene solution of crude 5-pregnene-3β,16β-diol-20-one ester (III) thus obtained was concentrated to 150 ml. and treated with 30 g. of potassium carbonate in 200 ml. of water. The two-phase mixture was heated to boiling and stirred rapidly for one-half hour. The benzene layer was separated, washed with salt water and evaporated to dryness. The residue was dissolved in 50 ml. of acetic anhydride, the solution refluxed for one hour, and the mixture chilled overnight. The crude crystalline pregnadienolone acetate (IV) was collected with suction, washed once with 25% acetic acid and four times with cold methanol and sucked dry. The residue on the filter was digested in 50 ml. of methanol for 15 minutes and chilled for two hours in an ice-bath. The product was collected, washed with cold methanol and dried. It melted at 167–170° and gave no depression of melting point upon fusion with pregnadienolone acetate of m.p. 170–173°; $[\alpha]^{25}D$ −40.6°; E, 246 (233 mμ) corresponding to 90% purity. The yield was 14.6 g. (37.3%).

An alternative hydrolysis was carried out by evaporating the benzene solution to dryness and treating with 90 ml. of methanol and 10 ml. of 10% hydrochloric acid. The solution was refluxed for two hours and extracted with ether. The ether solution was washed with water, bicarbonate solution and water. After being dried, the ether solution was

(10) D. K. Fukushima and T. F. Gallagher, THIS JOURNAL, 73, 196 1951).

(11) M. A. Saboor, *J. Chem. Soc.*, 922 (1945).

(12) Our thanks are due to Dr. William Tarpley and his staff for infrared spectra and to Mr. Edwin Conner and his staff for other physical measurements. Rotations are given for 1% solutions in chloroform. Ultraviolet absorptions were determined in isooctane as $E^{1\%}_{1cm.} = (1/cd) \log I_0/I$.

evaporated to dryness and the residue was acetylated as above.

(b) **Use of Hydrochloric Acid.**—A solution of 50 g. of crude I in 100 ml. of acetic anhydride was treated with 14.9 ml. (1 equiv.) of concd. hydrochloric acid. The mixture was refluxed for two hours, cooled and treated with 12.5 g. of sodium acetate. After oxidation, the reaction mixture was processed as above and gave 5.2 g. of pregnadienolone acetate (IV), m.p. 165–170°; $[\alpha]_D^{25}$ −39.5°; *E*, 264 (233 mμ) (96.0% pure).

(c) **Use of Acetyl Chloride.**—A sample of 50 g. of crude I in 100 ml. of acetic anhydride was treated with 8.55 g. (1 equiv.) of acetyl chloride. The mixture was refluxed for four hours, treated with 12.5 g. of sodium acetate and worked up as usual after oxidation. The product weighed 4.4 g., m.p. 165–171°; $[\alpha]_D^{24}$ −38.9°; *E*, 259 (233 mμ) (94.3% pure).

(d) **Use of *p*-Toluenesulfonic Acid.**—Fifty grams of crude I in 100 ml. of acetic anhydride was treated with 2 g. of *p*-toluenesulfonic acid and refluxed for eight hours. Sodium acetate (1 g.) was added and the mixture was oxidized and worked up as usual. Only 0.9 g. of pregnadienolone acetate (IV) was obtained, m.p. 170–173°; $[\alpha]_D^{23}$ −40.2°; *E*, 275 (233 mμ) 100% pure). The mother liquor was treated with water and gave 21.5 g. of resin which contained 2.3 g. of pregnadienolone acetate as indicated by the ultraviolet absorption maximum, *E*, 30 (235 mμ).

(e) **Other Catalysts.**—Undesired reactions occurred so that the product could not be isolated using the following acid catalysts: $SnCl_4$, BF_3, $ZnCl_2$, $HClO_4$, oxalic acid and trichloroacetic acid. There was little reaction with traces of acids. For example, 20 g. of I in 100 ml. of acetic anhydride was unaffected after refluxing with 0.1 cc. H_2SO_4 for eight hours.

Thermal Cleavage of 5-Pregnen-3β,16β-diol-20-one-3-acetate-16-(δ-acetoxy-γ-methylvalerate) (III).—Fifty grams of I was treated according to the directions (a) for the preparation of IV. After the reduction of excess chromic acid with sodium bisulfite solution, the oxidation mixture containing III was concentrated *in vacuo* and extracted with ether. The ether solution was washed neutral and dried over anhydrous magnesium sulfate. A sample evaporated to dryness showed *E*, 91 (237 mμ) corresponding to 33% of 5,16-pregnadien-3β-ol-20-one acetate (IV) (already formed due to cleavage under the acid conditions in the reaction and processing) in the crude III.

The ether solution was concentrated and mixed with 65 ml. of xylene. The solution was distilled until the temperature reached 135° and the remainder was refluxed for one hour. A sample was evaporated to dryness and showed *E*, 136.6 (237 mμ). This corresponds to 49.6% of IV, an increase of 50% over the pretreated mixture of III and IV.

The solution was chilled overnight and the product collected. The yield was 9.0 g., m.p. 156.2–165.6°; *E*, 185 (235 mμ) corresponding to a content of 67.5% of IV. Two recrystallizations from acetic anhydride gave 4.1 g. of pregnadienolone acetate, m.p. 170.5–172.5°; *E*, 269 (233 mμ); $[\alpha]_D$ −39.1°.

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NOTES

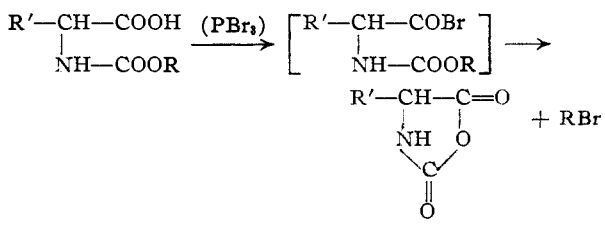
Synthesis of N-Carboxy-α-amino Acid Anhydrides from N-Carbalkoxy-α-amino Acids by the Use of Phosphorus Tribromide

BY DOV BEN-ISHAI AND EPHRAIM KATCHALSKI

RECEIVED FEBRUARY 6, 1952

The results of the investigation of the cyclization of N-carbalkoxy-β-haloalkylamines[1] and of the reaction between urethans and acetyl bromide and chloride,[2] suggested that N-carbalkoxy-α-amino acid bromides would cyclize more readily than the corresponding chlorides[3] to give N-carboxy-α-α-amino acid anhydrides.

It was, indeed, found that the N-carbalkoxy-α-amino acids given in Table I, when treated at room temperature with phosphorus tribromide, were converted with excellent yields, into the corresponding N-carboxy-α-amino acid anhydrides (oxazolidine-2,5-diones) (*cf.* Table II). The reaction is represented by the general equation



Under practically the same experimental conditions, N-carbethoxy- and N-carbobenzoxyanthranilic acid gave nearly quantitative yields of isatoic anhydride.

Experimental

N-Carbalkoxy-α-amino Acids.—The N-carbethoxy- and N-carbobenzoxyamino acids used were prepared by coupling ethyl chloroformate and carbobenzoxy chloride, respectively, with the corresponding α-amino acids in a manner similar to that prescribed for the synthesis of carbobenzoxyglycine.[4] The yields and analytical data are given in Table I.

N-Carboxy-α-amino Acid Anhydrides. General Procedure.—Phosphorus tribromide (0.02 mole) was added slowly to the N-carbalkoxy-α-amino acid (0.05 mole) dissolved or suspended in anhydrous ether (50 ml.). The reaction mixture was kept at room temperature for 12 hours. Dry petroleum ether (100 ml.) was added, and crystallization of anhydride promoted by keeping the reaction mixture for several hours at 4°. The anhydride, which separated out as a crystalline mass, was filtered, washed thoroughly with dry petroleum ether and recrystallized from a dry mixture of ethyl acetate and petroleum ether (Table II).

Isatoic Anhydride.—Phosphorus tribromide (0.035 mole) was added to 0.1 mole of N-carbethoxy or N-carbobenzoxyanthranilic acid (prepared by coupling anthranilic acid with the corresponding carbalkoxy chloride, in the usual way; *cf.* Table I) dissolved in anhydrous ether (100 ml.). After 24 hours at room temperature isatoic anhydride had separated as a microcrystalline product. It was filtered, washed with dry ether and recrystallized from alcohol; m.p. 240–243°,[5] yield about 90%.

Anal. Calcd. for $C_8H_5NO_3$: C, 58.9; H, 3.1; N, 8.6. Found: C, 58.5; H, 3.2; N, 8.3.

For further identification, the anhydride was converted

(1) E. Katchalski and D. Ben-Ishai, *J. Org. Chem.*, **15**, 1067 (1950).

(2) D. Ben-Ishai and E. Katchalski, *ibid.*, **16**, 1025 (1951).

(3) H. Leuchs, *Ber.*, **39**, 857 (1906); H. Leuchs and W. Geiger, *ibid.*, **41**, 1721 (1908); F. Wessely, *E. physiol. Chem.*, **146**, 72 (1925).

(4) "Organic Syntheses," **23**, 14 (1943).

(5) E. Erdmann, *Ber.*, **22**, 2165 (1889), gives m.p. 240° (dec).