prisms (0.42 g., 40%), m.p. 283°, mixed m.p. with 10-phenoxy-9,10-dihydro-9,10-azaphosphaphenanthrene 10-oxide, 244–250°. The same compound was isolated in some cases from the mother liquors from the recrystallization of II (R = Ph).

Anal. Calcd. for $C_{18}H_{14}ONP$: C, 74.2; H, 4.8; N, 4.8; P, 10.7. Found: C, 74.3; H, 4.9; N, 5.0; P, 10.9.

9,10-Dihydro-9,10-azaphosphaphenanthrene 10-Oxide.—Crude 10-chloro-9,10-dihydro-9,10-azaphosphaphenanthrene (6 g.) was shaken with water and methylene chloride. The residue from evaporation of the organic layer crystallized from methylene chloride in stout white prisms (3.9 g., 71%), m.p. 193–194°.

Anal. Calcd. for $C_{12}H_{10}ONP$: C, 67.0; H, 4.6; N, 6.5; P, 14.4. Found: C, 67.1; H, 4.6; N, 6.5; P, 14.0.

10-Methyl-9,10-dihydro-9,10-azaphosphaphenanthrene 10-Methiodide (VII).—To a solution of crude 10-chloro-9,10-dihydro-9:10-azaphosphaphenanthrene, prepared from 2-aminobiphenyl (8.2 g.), in methylene chloride (500 ml.) was added one of methylmagnesium iodide, prepared from magnesium (1.8 g.) and methyl iodide (10.3 g.) in ether. Methyl iodide (10.5 g.) was then added and the solution boiled under reflux for 8 hours. Ice was added and the residue from evaporation of the organic layer crystallized from methylene chloride in light yellow prisms (11.2 g., 65%), m.p. 230–233° dec.

Anal. Calcd. for $C_{14}H_{15}NPI$: C, 47.3; H, 4.2; N, 3.9; P, 8.7; I, 35.8. Found: C, 47.5; H, 4.3; N, 3.9; P, 9.0; I, 35.4.

10-Phenyl-9,10-dihydro-9,10-azaphosphaphenanthrene Methiodide (VIII).—A solution of 10-phenyl-9,10-dihydro-9,10-azaphosphaphenanthrene (1 g.) and methyl iodide (1.9 g.) in dry benzene (100 ml.) was boiled under reflux for 6 hours when X separated in almost theoretical yield. It crystallized from methylene chloride in light yellow prisms, m.p. 214°.

Anal. Calcd. for $C_{19}H_{17}NPI$: C, 54.6; H, 4.1; N, 3.4; P, 7.4; I, 30.4. Found: C, 54.7; H, 4.1; N, 3.3; P, 7.2; I, 30.1.

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[CONTRIBUTION FROM THE RESEARCH DEPARTMENT, CIBA PHARMACEUTICAL PRODUCTS, INC., SUMMIT, NEW JERSEY]

Some Hypotensive Amino Steroid Glycosides[1]

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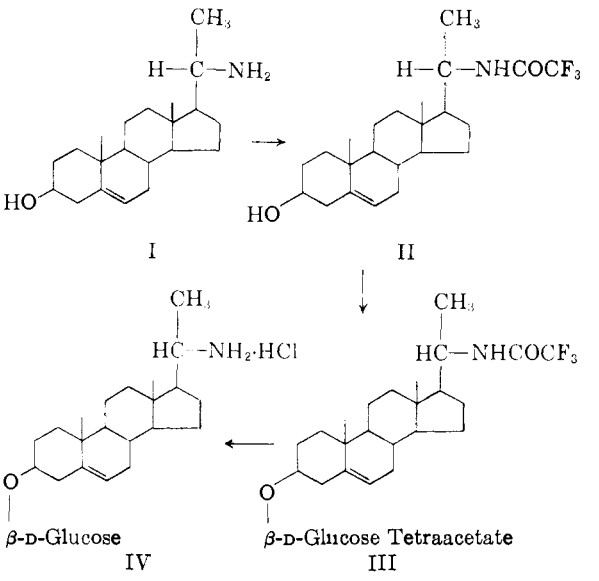
A new hypotensive amino steroid glycoside has been isolated from the roots of *Conopharyngia pachysiphon*. Degradation experiments have demonstrated that this substance is 20α-amino-5-pregnen-3β-yl β-D-glucoside hydrochloride. This has been confirmed by synthesis and a number of related compounds have been prepared. Several of these substances have shown considerable hypotensive activity on intravenous administration.

In a previous communication[2] we reported the isolation of the substance responsible for the hypotensive action of a methanol extract of the roots of *Conopharyngia pachysiphon*.[3] It was found to have the empirical formula $C_{27}H_{45}NO_6 \cdot HCl$. Acid hydrolysis yielded two aglycons which were identified as 20α-amino-5-pregnen-3β-ol and its dehydration product, 3,5-pregnadiene-20α-amine. The sugar moiety also resulting from this hydrolysis was shown to be D-glucose. On the basis of these data the active substance was assigned the structure 20α-amino-5-pregnen-3β-yl β-D-glucose hydrochloride (IV). This was confirmed by the synthesis outlined in chart I.

In spite of the high intravenous activity of IV (Table I) it demonstrated little if any hypotensive effect on oral administration. This fact led us to synthesize a number of related amino steroid glycosides in the hope that an effective orally active substance could be produced. In general they were prepared by suitable modification of the above synthetic scheme and the results are tabulated in Table I. It is evident that in no case was our goal achieved although several of the products were quite active when given intravenously.

CHART I



Further paper chromatographic study of *C. pachysiphon* root and bark extracts has demonstrated the presence of several other alkaloids. However, none of these on preliminary examination have shown activity nor do they seem to resemble chemically those recently reported from *C. durissima*.[4] They are now under further investigation.

(1) Presented in part before the Division of Medicinal Chemistry at the Atlantic City Meeting of the American Chemical Society, September 15, 1959.

(2) D. Dickel, R. Lucas and H. MacPhillamy, THIS JOURNAL, **81**, 3154 (1959).

(3) Prof. F. J. Simmonds, Imperial College of Tropical Agriculture, Trinidad, B.W.I., has recently informed us that this material originally came from the Island of Principé, Portugese West Africa.

(4) U. Renner, D. A. Prins and W. G. Stoll, *Helv. Chim. Acta*, **42**, 1572 (1959).

TABLE I

DERIVATIVES OF 20-AMINOPREGNAN-3β-OL

	C_3	C_5	C_{20}	Activity—I.v.[a]	Activity—Intestine[b]
I	....	Δ^5	α	2.0	..
IV	β-D-Glucoside	Δ^5	α	0.04	15
V	β-D-Glucoside	Δ^5	β	.2	10
VI	β-D-Glucoside	α	α	.07	5
VII	β-D-Glucoside	α	β	.2	5
VIII	α-D-Glucoside	α	α	3.0	15
IX	L-Rhamnoside	Δ^5	β	1.0	..
X	α-L-Arabinoside	Δ^5	β	0.5	10
XI	Lactoside	α	α	3.0	15
XII	β-D-Glucoside	α	β-Dimethyl	..	15
XIII	β-D-Tetramethyl glucoside	Δ^5	α	..	10
XIV	Sodium glucuronate	α	α	..	15
XV	3,4,5-Trimethoxybenzoyl	Δ^5	β	>5.0	..
XVI	Hemisuccinoyl	α	α	..	15
	DERIVATIVES OF 17-AMINOANDROSTAN-3β-OL				
XVII	β-D-Glucoside	Δ^5		1.0	5
XVIII	β-D-Glucoside	α		..	15
XIX	α-L-Arabinoside triacetate	α		..	15
XX	α-L-Arabinoside	α		..	15

[a] With the exception of XIII and XIV all compounds were tested as the hydrochlorides. The activity is expressed as the dose in mg. per kg. of animal which produces a lowering of the mean arterial blood pressure of 60 mm. or more. [b] Significant activity was not observed when this maximum dose was administered into the gut.

Acknowledgments.—We wish to express our thanks to Dr. E. Schlittler for his interest and encouragement during this investigation. We greatly appreciate the help given to us by Mr. B. Korzun and his associates in the paper chromatographic laboratory and by the personnel of the microanalytical and spectral laboratories. We are especially anxious to thank Mr. Louis Dorfman for making many valuable suggestions and spectral interpretations. We also wish to acknowledge the close coöperation of Dr. A. Plummer, Dr. W. Barrett and their associates in our Macrobiology Division who determined the biological activity of our preparations.

Experimental[5]

Isolation Procedure.—To 4.5 kg. of ground *Conopharyngia pachysiphon* roots was added 25 l. of methanol and the mixture was refluxed for 1 hr. The hot extract was then removed by filtration. This process was repeated three times with 24, 15 and 15 l. portions of solvent. Evaporation of the combined extracts yielded about 300 g. of residue.

Since the extract was not completely soluble in 1 l. of benzene 300 g. of Filter Cel was added to form a uniform slurry. This mixture was then poured onto a column of 1600 g. silicic acid (Mallinckrodt, 100 mesh). Fractions (Table II) were taken, during which time the eluting solvents were allowed to percolate through the slurry at the top of the column. In this way practically all of the root extract was dissolved and passed through the column. The total time required was about 28 days. The eluates were evaporated to dryness *in vacuo*.

(5) All melting points were taken in a capillary tube in an electrically heated aluminum block and are uncorrected. The infrared curves were made with a Perkin-Elmer model-21 spectrophotometer and the optical rotations were measured in chloroform unless otherwise stated.

TABLE II

Eluate	Solvent	Volume (l.)	Weight (g.)
1	Benzene	8.0	10.2
2–3	Chloroform	8.0	1.7
4–9	$CHCl_3$(5):MeOH(1)	14.5	120.4
10–16	$CHCl_3$(1):MeOH(1)	15.5	111.7
17–19	Methanol	11.0	49.8

Pharmacological evaluation indicated that the hypotensive activity was in fractions 10–16. Upon the addition of ethanol to each of these fractions crystals formed. They were removed by filtration and yielded a total of 17.6 g. of crude crystalline product, m.p. 230–240°. Repeated recrystallization from ethanol produced the pure active material (IV), m.p. 259–260°, $[\alpha]_D^{25}$ −66.6° (pyridine 90%: water 10%).

Anal. Calcd. for $C_{27}H_{46}ClNO_6$: C, 62.83; H, 8.98; N, 2.71; Cl, 6.87. Found: C, 62.71; H, 9.13; N, 2.62; Cl, 6.96. Van Slyke amino N analysis[6]: Calcd. 2.71. Found: 2.61.

Fractions 4–9 contained most of the material responsible for the respiratory depressant action also present in the original extract. From fractions 7–9 there was obtained 760 mg. of a steroid amine later identified as 20α-amino-5-pregnen-3β-ol hydrochloride (I).

The base of the glycoside was obtained by dissolving the above hydrochloride IV in aqueous ethanol and adding an excess of dilute ammonium hydroxide. On standing, the base crystallized from solution. It was filtered off and washed well with ethanol, m.p. 285–288°. Its insolubility in most solvents made recrystallization impractical; γ_{max}^{Nujol} 3528, 3403, 3270, 1069 and 1015 cm.$^{-1}$.

Anal. Calcd. for $C_{27}H_{45}NO_6$: C, 67.61; H, 9.46; N, 2.92. Found: C, 67.12; H, 9.29; N, 3.19.

20α-Acetamido-5-pregnen-3β-yl β-D-Glucoside Tetraacetate.—A suspension of 60 mg. of the above base in 5 ml. of acetic anhydride and 3 ml. of pyridine was warmed on the steam-bath for 5–10 minutes until the solid material dissolved. After standing 1 hr. at room temperature the solution was evaporated *in vacuo* to dryness. A small amount of ethanol containing a few drops of ammonium hydroxide was then added and the mixture re-evaporated. Finally the residue was dissolved in ethanol and crystallization induced by the careful addition of water. After recrystallization from the same solvent 55 mg. of product was obtained, m.p. 200–203°; γ_{max}^{Nujol} 1655, 1750, 1069 and 1015 cm.$^{-1}$.

Anal. Calcd. for $C_{37}H_{55}NO_{11}$: C, 64.42; H, 8.04; N, 2.03; 4-Acetyl, 24.96. Found: C, 63.87; H, 8.14; N, 2.14; Acetyl, 25.3.

20α-Acetamido-5-pregnen-3β-yl β-D-Glucoside.—A solution of 300 mg. of the above pentaacetate and 340 mg. of potassium hydroxide in 30 ml. of methanol was refluxed for 6 hr. The mixture was then cooled, diluted with an equal volume of water and evaporated to one half volume *in vacuo* during which time a white crystalline product formed. After adjustment of the solution to *p*H 6 with acetic acid, the product was removed by filtration and washed with water. The crude substance (205 mg.) was recrystallized from ethanol–water, ethanol–acetone and finally from ethanol to obtain an analytically pure sample, m.p. 289–292°. γ_{max}^{Nujol} 3459, 3303, 1658 and 1565 cm.$^{-1}$.

Anal. Calcd. for $C_{29}H_{47}NO_7$: C, 66.76; H, 9.08; N, 2.68. Found: C, 66.40; H, 9.11; N, 2.70.

Hydrolysis of 20α-Amino-5-pregnen-3β-yl β-D-Glucoside Hydrochloride.—A mixture of 4.0 g. of isolated (IV), 200 ml. of acetic acid, 200 ml. of ethanol and 200 ml. of hydrochloric acid (6 *N*) was refluxed for 11 hr. The acidic solution was then concentrated to a small volume *in vacuo* and diluted with 100 ml. of water. After making the resulting suspension alkaline with potassium hydroxide, the organic material was extracted with methylene chloride. The extract was washed with water, dried and the solvent removed leaving 2.4 g. of residue. This was chromatographed on 40 g. of Woelm neutral alumina (activity II–III) in benzene solution.

From the benzene eluate there was obtained 1.14 g. of a substance identified by its analysis and by its ultraviolet and

(6) This determination was made by the Schwarzkopf Microanalytical Laboratory.

infrared spectra as 3,5-pregnadiene-20α-amine, m.p. 111–115°, after recrystallization from petroleum ether (30–60°). $[\alpha]^{25}D$ −102.6°, λ_{max}^{EtOH} 230 shoulder 234, 243 shoulder mμ. (ε 15,260; 16,700; 12,190); γ_{max}^{Nujol} 3352, 3276, 1649 and 1600 cm.$^{-1}$.

Anal. Calcd. for $C_{21}H_{33}N$: C, 84.22; H, 11.11; N, 4.68. Found: C, 83.76; H, 11.07; N, 4.64.

The methylene chloride–methanol (90:10) eluate of the above chromatogram yielded 100 mg. of a second substance identified as 20α-amino-5-pregnen-3β-ol (I),[7] m.p. 168–173° after recrystallization from ethyl acetate; γ_{max}^{Nujol} 3330, 3170, 1670 and 1600 cm.$^{-1}$.

Anal. Calcd. for $C_{21}H_{35}NO$: C, 79.44; H, 11.11; N, 4.41. Found: C, 78.84; H, 10.99; N, 4.33.

Isolation and Identification of Glucose.—The reaction mixture resulting from the hydrolysis of 2.0 g. of active glycoside hydrochloride according to the acetic acid–hydrochloric acid procedure described above was evaporated to dryness *in vacuo*. Repeated addition and evaporation of 10 ml. portions of water removed most of the volatile acids. The resulting residue was taken up in 100 ml. of water and insoluble steroidal material was removed by filtration. The resulting aqueous solution was passed through a 50 cm. column of Amberlite IR-45 anion exchange resin. The eluate and the water washings of the column were evaporated *in vacuo* to a syrup (650 mg). which could not be crystallized. A portion of this syrup was identified as D-glucose by paper chromatography.[8]

As a further identification of the sugar a 400 mg. sample of the above syrup was oxidized to potassium gluconate and then treated with *o*-phenylenediamine.[9] The resulting benzimidazole, m.p. 210–220°, gave an infrared absorption curve identical with that obtained from an authentic sample of D-glucose. $[\alpha]^{25}D$ +8.5° (5% citric acid), reported,[9] $[\alpha]^{25}D$ +9.6°.

20α-Amino-5-pregnen-3β-ol (I).—A solution of 7.0 g. of 20α-amino-5-pregnen-3β-ol acetate[10] in 100 ml. of 2% methanolic potassium hydroxide was refluxed for 2 hr. The alkaline mixture was poured into an excess of water and the amino alcohol extracted with ether. The ether solution was washed with water, dried with sodium sulfate and the solvent removed leaving 5.7 g. of product, m.p. 164–167°. Recrystallization from ethyl acetate yielded an analytical sample, m.p. 172–174°, $[\alpha]^{25}D$ −69.2°.

Anal. Calcd. for $C_{21}H_{35}NO$: C, 79.49; H, 11.11; N, 4.41. Found: C, 79.30; H, 11.20; N, 4.48.

20α-Trifluoroacetamido-5-pregnen-3β-ol (II).—To a suspension of 3.56 g. of the above hydroxy amine I in 26 ml. of dry pyridine was added portionwise 8.37 g. of trifluoroacetic anhydride.[11] A gum formed which soon went into solution and the reaction mixture was allowed to stand for 2 hr. at room temperature. At the end of this time it was poured into ice and water and the product extracted with ether. The ether solution was washed with water and then dried with sodium sulfate. After removal of the solvent 5.75 g. of yellow solid remained which consisted of a mixture of mono and diacylated material.

This was partially saponified by dissolving it in 200 ml. of warm 95% ethanol, cooling to room temperature and adding 7.3 g. of potassium bicarbonate in 100 ml. of water. The resulting suspension was allowed to stand at room temperature for 24 hr. and then diluted with an excess of water. The precipitated material was extracted with chloroform and the organic phase was washed with water and dried. Removal of the solvent *in vacuo* yielded 3.46 g. of product m.p. 196–200°. An analytical sample prepared by recrystallization from methanol melted 199–201°.

Anal. Calcd. for $C_{23}H_{34}F_3NO_2$: C, 66.80; H, 8.29. Found: C, 66.59; H, 8.31.

20α-Trifluoroacetamido-5-pregnen-3β-yl β-D-Glucoside Tetraacetate. (III).—To a solution of 2.96 g. of the above compound II in 50 ml. of dry chloroform was added 5.92 g. of acetobromoglucose, 5.92 g. of freshly prepared silver oxide and 5.92 g. of pulverized Drierite. The mixture was stirred at room temperature for 24 hr. and then the insoluble material was removed by filtration. Evaporation of the solvent yielded 8.3 g. of a colorless glass which could not be crystallized. Therefore, 5.5 g. of this material was chromatographed on 150 g. of Woelm neutral alumina in benzene solution. The fractions eluted with 20% acetone in benzene yielded 3.45 g. of crystalline product, m.p. 198–204°. An analytical sample was prepared by recrystallization from ethyl acetate–hexane, m.p. 200–205°.

Anal. Calcd. for $C_{37}H_{52}F_3NO_{11}$: C, 59.75; H, 7.05. Found: C, 59.66; H, 7.14.

20α-Amino-5-pregnen-3β-yl β-D-Glucoside.—A mixture of 3.0 g. of the crystalline material mentioned above (III) in 100 ml. of 95% ethanol and 15 ml. of 10% sodium hydroxide solution was refluxed for 3 hr. On cooling 650 mg. of crystalline material separated. An additional 420 mg. was obtained by concentration of the mother liquor for a total of 1.07 g., m.p. 278–280°. This material was difficultly soluble and a satisfactory recrystallization solvent could not be found. However, it was further purified by dissolving it in a large volume of methanol, filtering the hot solution and concentrating the filtrate to a small volume. The crystals which separated melted 285–287°.

Anal. Calcd. for $C_{27}H_{45}NO_6$: C, 67.61; H, 9.46. Found: C, 67.16; H, 9.44.

20α-Amino-5-pregnen-3β-yl β-D-Glucoside Hydrochloride (IV).—A suspension of 120 mg. of the above base in 10 ml. of 95% ethanol containing four drops of 6 *N* hydrochloric acid was gently warmed on the steam-bath. Sufficient water was carefully added dropwise until the material went into solution. On cooling the hydrochloride crystallized, m.p. 257–259°.

Anal. Calcd. for $C_{27}H_{46}ClNO_6$: C, 62.83; H, 8.98. Found: C, 62.53; H, 8.88.

This material was identical in every respect with that isolated from the root extract.

20β-Amino-5-pregnen-3β-ol.—To a refluxing solution of 21.5 g. of 3β-hydroxy-5-pregnen-20-one oxime[12] in 1.1 l. of dry *n*-propanol was added 34.2 g. of sodium in small pieces over a period of 1 hr. The mixture was refluxed until all the sodium had dissolved. Approximately 400 ml. of propanol was removed *in vacuo* at 100° and the cooled residue poured into 2 l. of water. The resulting solution or suspension was extracted four times with 500 ml. portions of ethyl acetate. The combined extracts were washed with water, the solution concentrated to 750 ml. and then dried overnight with anhydrous sodium sulfate. The drying agent was removed and 500 ml. of dry ether saturated with dry hydrogen chloride was added. The resulting hydrochloride was removed by filtration and the crystals washed immediately with dry ether. In this way 15.0 g. of product, m.p. 308–311°, was obtained.

To convert the hydrochloride to the base, the above material was dissolved in a hot mixture of 225 ml. of water and 100 ml. of 95% ethanol and then 35 ml. of 10% sodium hydroxide was added. The precipitated base was extracted with ethyl acetate, the organic phase dried and on removal of the solvent 12.5 g. of amine remained, m.p. 163–166°. After repeated recrystallization from methanol and finally from ethyl acetate an analytical sample was obtained, m.p. 170–172°, $[\alpha]^{25}D$ −60.4°.

Anal. Calcd. for $C_{21}H_{35}NO$: C, 79.44; H, 11.11; N, 4.41. Found: C, 79.12; H, 11.42; N, 4.39.

A mixture of this material and the 20α-epimer melted at 170–171° and their respective infrared absorption curves were indistinguishable.

20β-Trifluoroacetamido-5-pregnen-3β-ol.—A 5.8 g. sample of the above 20β-aminosteroid was acylated with trifluoroacetic anhydride according to directions given for the preparation of II. After recrystallization from methanol 3.5 g. of product was obtained, m.p. 181–182°.

(7) It is very difficult, if not impossible, to distinguish most of the 20-amino steroid epimers by m.p., by the depression of the m.p. of mixtures or by differences in their respective infrared absorption curves in solution or as a Nujol mull. An exception is the pair of 20-trifluoroacetamido-5-pregnen-3β-ol epimers described later. This derivative provided the best proof that the isolated amino steroid hydrolysis product was in fact the 20α-epimer.

(8) S. Partridge, *Nature*, **164**, 443 (1949); J. Cerbulis, *Anal. Chem.*, **27**, 1400 (1955).

(9) S. Moore and K. P. Link, *J. Biol. Chem.*, **133**, 293 (1940).

(10) P. L. Julian, E. W. Meyer and H. C. Printy, THIS JOURNAL, **70**, 887 (1948).

(11) A. Lardon and T. Reichstein, *Helv. Chim. Acta*, **37**, 443 (1954).

(12) A. Butenandt and V. Westphal, *Ber.*, **69**, 443 (1936).

Anal. Calcd. for $C_{23}H_{34}F_3NO_2$: C, 66.80; H, 8.3. Found: C, 66.66; H, 8.34.

The melting point of this substance was significantly lower than the corresponding 20α-isomer (II) and their infrared curves were easily distinguishable.

20β-Trifluoroacetamido-5-pregnen-3β-yl β-D-Glucoside Tetraacetate.—The above 20β-trifluoroamide (2.8 g.) was converted to the glucoside acetate by the method used for the preparation of III; yield 2.1 g., m.p. 205–206°.

Anal. Calcd. for $C_{37}H_{52}F_3NO_{11}$: C, 59.75; H, 7.05. Found: C, 59.56; H, 7.17.

20β-Amino-5-pregnen-3β-yl β-D-Glucoside Hydrochloride (V).—Hydrolysis of 1.5 g. of the above tetraacetate according to the method used for the preparation of the corresponding 20α-epimer yielded 450 mg. of crude base glucoside, m.p. 293–295°.

The base was converted to the hydrochloride as for IV, m.p. 264–267°.

Anal. Calcd. for $C_{27}H_{46}ClNO_6$: C, 62.83; H, 8.98; N, 2.71. Found: C, 62.76; H, 9.09; N, 2.87.

20β-Trifluoroacetamido-5-pregnen-3β-yl β-L-Rhamnoside Triacetate.—Two g. of 20β-trifluoroacetamido-5-pregnen-3β-ol was treated with 5.0 g. of acetobromorhamnose[13] according to the procedure used for the synthesis of III. The yield was 1.4 g. of crude product, m.p. 109–115°. This material was used directly in the next step.

20β-Amino-5-pregnen-3β-yl β-L-Rhamnoside Hydrochloride (IX).—The above triacetate (1.0 g.) was hydrolyzed and the resulting rhamnoside base converted directly to the hydrochloride as described for the preparation of IV. Yield 230 mg., m.p. 275–285°.

Anal. Calcd. for $C_{27}H_{46}ClNO_5$: C, 64.84; H, 9.27; N, 2.80; Cl, 7.09. Found: C, 65.36; H, 9.53; N, 3.02; Cl, 7.45.

20β-Trifluoroacetamido-5-pregnen-3β-yl α-L-Arabinoside Triacetate.—Two grams of 20β-trifluoroacetamido-5-pregnen-3β-ol was reacted in the usual way with acetobromarabinose[14] and 980 mg. of product was obtained, m.p. 110–118°.

Anal. Calcd. for $C_{34}H_{46}F_3NO_9$: C, 60.79; H, 7.20; N, 2.08. Found: C, 60.49; H, 7.33; N, 2.33.

20β-Amino-5-pregnen-3β-yl α-L-Arabinoside Hydrochloride (X).—The hydrolysis of 930 mg. of the above material yielded 400 mg. of base. This was converted directly to the hydrochloride, m.p. 232–234°, by the procedure already described for IV.

Anal. Calcd. for $C_{26}H_{44}ClNO_5$: C, 64.24; H, 9.12; N, 2.88; Cl, 7.29. Found: C, 63.76; H, 9.38; N, 2.96; Cl, 7.42.

20α-Amino-5α-pregnan-3β-ol.—A solution of 500 mg. of 20α-amino-5-pregnen-3β-ol in 500 ml. of acetic acid was catalytically reduced at atmospheric pressure using 50 mg. of platinum oxide catalyst. After the theoretical amount of hydrogen had been taken up, the catalyst was removed and the solution concentrated to dryness *in vacuo*. The residue was dissolved in warm aqueous methanol and made alkaline with 10% sodium hydroxide solution. The precipitated base was filtered off and recrystallized from ethanol–water, 350 mg., m.p. 174–175°.

Anal. Calcd. for $C_{21}H_{37}NO$: C, 78.94; H, 11.67; N, 4.38. Found: C, 78.61; H, 11.50; N, 4.59.

20β-Amino-5α-pregnan-3β-ol.—In a similar manner 5.0 g. of 20β-amino-5-pregnen-3β-ol was reduced to yield 3.88 g. saturated amine, m.p. 174–176°. A mixed melting point with the 20α-epimer showed no depression.

Anal. Calcd. for $C_{21}H_{37}NO$: C, 78.94; H, 11.67; N, 4.38. Found: C, 79.24; H, 12.04; N, 4.25.

20α-Trifluoroacetamido-5α-pregnan-3β-ol.—The acylation of 350 mg. of 20α-amino-5α-pregnan-3β-ol according to the previously described method for II resulted in the formation of 350 mg. of product, m.p. 214–215°.

Anal. Calcd. for $C_{23}H_{36}F_3NO_2$: C, 66.48; H, 8.73. Found: C, 66.10; H, 8.73.

20β-Trifluoroacetamido-5α-pregnan-3β-ol.—A 3.88 g. sample of the 20β-amino compound similarly yielded 4.0 g. of product, m.p. 219–221°. The m.p. of a mixture with the 20α-epimer was 195°.

Anal. Calcd. for $C_{23}H_{36}F_3NO_2$: C, 66.48; H, 8.73. Found: C, 65.90; H, 8.66.

The infrared absorption curve of this substance in solution was very similar to that of the above 20α-epimer, however, there were certain minor but definite differences in the 800–1000 cm.$^{-1}$ region.

20α-Trifluoroacetamido-5α-pregnan-3β-yl β-D-Glucoside Tetraacetate.—The reaction of 350 mg. of the 20α-isomer above with acetobromglucose in the usual manner yielded 500 mg. of oil which was purified by chromatography. The final product, 150 mg., melted 218–220°.

Anal. Calcd. for $C_{37}H_{54}F_3NO_{11}$: C, 59.58; H, 7.30. Found: C, 59.27; H, 7.70.

20β-Trifluoroacetamido-5α-pregnan-3β-yl β-D-Glucoside Tetraacetate.—In a similar way 1.7 g. of the 20β-epimer yielded 480 mg. of product, m.p. 218–220°.

Anal. Calcd. for $C_{37}H_{54}F_3NO_{11}$: C, 59.58; H, 7.30. Found: 59.20; H, 7.51.

20α-Amino-5α-pregnan-3β-yl β-D-Glucoside Hydrochloride (VI).—The hydrolysis of 720 mg. of the above 20α-glycoside acetate yielded 350 mg. of glycoside base, m.p. 255–266°. This was not purified further but was converted to the hydrochloride by the standard method, 250 mg., m.p. 250–252°.

Anal. Calcd. for $C_{27}H_{48}ClNO_6$: C, 62.59; H, 9.34; N, 2.70. Found: C, 62.88; H, 9.63; N, 2.58.

20β-Amino-5α-pregnen-3β-yl β-D-Glucoside Hydrochloride (VII).—The 20β-epimer, 1.95 g., was hydrolyzed in the same manner to yield 950 mg. of crystalline base, m.p. 243–245°. The hydrochloride, 850 mg., melted at 240–241°.

Anal. Calcd. for $C_{27}H_{48}ClNO_6$: C, 62.59; H, 9.34; N, 2.70. Found: C, 61.98; H, 9.49; N, 2.74.

20-Keto-5α-pregnan-3β-yl α-D-Glucoside Tetraacetate.—A solution of 1.53 g. of 3β-hydroxy-5α-pregnan-20-one[15] in 40 ml. of benzene was refluxed for 5 hr. with 2.0 g. of acetobromglucose and 800 mg. of mercuric acetate.[16] The solution was then washed with water, dried and the solvent removed *in vacuo*. The residue was recrystallized from ethanol and yielded 400 mg. of product, m.p. 205–207°.

Anal. Calcd. for $C_{35}H_{52}O_{11}$: C, 64.80; H, 8.08. Found: C, 64.71; H, 7.94.

Oxime.—A solution of 3.58 g. of the above ketone in 36 ml. of pyridine was heated on the steam-bath for 5 hr. with 813 mg. of hydroxylamine hydrochloride. The mixture was cooled, poured into ice and water and the precipitated material filtered off and washed with water. After recrystallization from methanol there was obtained 2.19 g. of oxime, m.p. 212–214°.

Anal. Calcd. for $C_{35}H_{53}NO_{11}$: C, 63.33; H, 8.05; N, 2.11. Found: C, 63.70; H, 8.06; N, 2.15.

20α-Amino-5α-pregnan-3β-yl α-D-Glucoside Hydrochloride (VIII).—A 2.19 g. sample of the above oxime was catalytically reduced in acetic acid solution using 550 mg. of platinum oxide catalyst at atmospheric pressure. After removing the catalyst and concentrating the solvent, the material was hydrolyzed by refluxing it in a mixture of 25 ml. of ethanol, 5 ml. of water and 2.0 g. of sodium hydroxide for 2 hr. It was then poured into cold water and the precipitated material removed by filtration. It was then dissolved in ethanol containing an excess of hydrochloric acid. On concentrating the solution *in vacuo* at 40° crystals separated and after recrystallization from ethanol yielded 430 mg. of material, m.p. 259–260°.

Anal. Calcd. for $C_{27}H_{48}ClNO_6$: C, 62.59; H, 9.34; N, 2.70. Found: C, 62.27; H, 9.58; N, 2.85.

20α-Amino-5α-pregnan-3β-yl Lactoside Hydrochloride (XI).—Four g. of 3β-hydroxy-5α-pregnan-20-one was treated with 12.0 g. of acetobromlactose[17] in the usual manner as for the preparation of (III). The resulting crude noncrystalline product (7.5 g.) was converted to the oxime in pyridine solution as previously described and yielded 2.8 g.

(13) E. Fischer, M. Bergmann and A. Rabe, *Ber.*, **53**, 2372 (1920).
(14) M. Gehrke and F. Aichner, *ibid.*, **60B**, 918 (1927).
(15) P. A. Plattner, H. Heusser and E. Angliker, *Helv. Chim. Acta*, **29**, 468 (1946).
(16) R. E. Marker and J. Krueger, THIS JOURNAL, **62**, 3349 (1940).
(17) G. Zemplén. Z. Csürös and Z. Bruckner, *Ber.*, **61**, 927 (1928).

of crude material. This was directly hydrogenated to produce 2.15 g. of amine which was not isolated. After hydrolysis with barium methylate in methanol[18] the product was converted to the hydrochloride, 400 mg., m.p. 253–258°.

Anal. Calcd. for $C_{33}H_{58}ClNO_{11}$: C, 58.26; H, 8.59; N, 2.06; Cl, 5.21. Found: C, 58.64; H, 8.37; N, 1.91; Cl, 4.68.

20-Keto-5α-pregnan-3β-yl β-(D-Glucuronide Methyl Ester Triacetate).—A mixture of 4.5 g. of 3β-hydroxy-5α-pregnan-20-one, 6.0 g. of methyl acetobromglucuronate,[19] 8.0 g. of silver oxide and 8.0 g. of anhydrous calcium sulfate in 50 ml. of dry chloroform was stirred for 24 hr. and then worked up as usual. This resulted in the isolation of 3.45 g. of product which after recrystallization from ethanol melted at 201–202°.

Anal. Calcd. for $C_{34}H_{50}O_{11}$: C, 64.33; H, 7.95. Found: C, 64.09; H, 7.91.

Oxime.—The above ketone (3.0 g.) was converted to the oxime by heating with hydroxylamine hydrochloride in pyridine solution as previously described; Yield 2.35 g., m.p. 184–187°.

Anal. Calcd. for $C_{34}H_{51}NO_{11}$: C, 62.80; H, 7.91; N, 2.16. Found: C, 63.07; H, 8.09; N, 2.23.

20α-Amino-5α-pregnan-3β-yl β-(D-Glucuronide Methyl Ester Triacetate).—The above oxime was reduced catalytically in acetic acid and yielded 1.50 g. of material, m.p. 178–179°.

Anal. Calcd. for $C_{34}H_{53}NO_{10}$: C, 64.21; H, 8.40; N, 2.12. Found: C, 64.51; H, 8.46; N, 2.19.

20α-Amino-5α-pregnan-3β-yl β-(D-Glucuronide Sodium Salt) (XIV).—The saponification of 1.45 g. of the above ester with methanolic sodium hydroxide yielded 750 mg. of sodium salt. This was recrystallized from ethanol–water containing a few drops of 10% sodium hydroxide solution, m.p. > 300° (dec.).

Anal. Calcd. for $C_{27}H_{44}NaNO_7$: C, 62.65; H, 8.57; N, 2.70. Found: C, 62.75; H, 8.82; N, 2.65.

Acetate Salt of 20α-Amino-5-pregnen-3β-yl β-D-Tetramethylglucoside (XIII).—Bromotetramethyl-D-glucose[20] (16.0 g.) was allowed to react with 3β-hydroxy-5-pregnen-20-one (10.0 g.) in the manner previously described for glycoside formation of III. The material (5.0 g.) was recrystallized from ethanol, m.p. 139–142°.

Oxime.—The above ketone glycoside (4.5 g.) was converted to the oxime in pyridine solution yielding 3.4 g. of product, m.p. 185–188°.

Hydrogenation of 3.0 g. of oxime gave 710 mg. of purified acetate salt, m.p. 183–188°, after recrystallization from ethyl acetate.

Anal. Calcd. for $C_{33}H_{57}NO_8$: C, 66.52; H, 9.64; N, 2.35. Found: C, 67.17; H, 10.09; N, 2.53.

3β-Hydroxy-5α-pregnan-20-one 3-Hemisuccinate.—To a solution of 1.67 g. of 3β-hydroxy-5α-pregnan-20-one in 15 ml. of pyridine was added 500 mg. of succinic anhydride. The mixture was allowed to stand 24 hr. at room temperature and then it was slowly poured into ice-cold 20% sulfuric acid solution. The crystals were removed by filtration, dried and recrystallized from ethanol, 1.5 g., m.p. 203–205°.

Anal. Calcd. for $C_{25}H_{38}O_5$: C, 71.74; H, 9.15. Found: C, 71.77; H, 9.31.

Oxime.—The above compound (1.4 g.) was refluxed for 4 hr. in 60 ml. of ethanol containing 330 mg. of hydroxylamine hydrochloride and 450 mg. of sodium acetate. The mixture was then diluted with water, the product filtered off and recrystallized from ethanol, 1.03 g., m.p. 220–221°.

Anal. Calcd. for $C_{25}H_{39}NO_5$: C, 69.25; H, 9.07. Found: C, 69.07; H, 9.03.

20α-Amino-5α-pregnan-3β-ol 3-Hemisuccinate Hydrochloride (XVI).—One g. of the above oxime was catalytically hydrogenated as previously described for VIII. The crystalline acetate salt obtained on evaporation of the solvent, m.p. 268–269°, was dissolved in hot ethanol and a few drops of dilute hydrochloric acid added. On concentrating the solution, crystals separated and after recrystallization from ethanol yielded 825 mg. of hydrochloride, m.p. 310–313° (dec.).

Anal. Calcd. for $C_{25}H_{42}ClNO_4$: C, 65.83; H, 9.28; N, 3.07. Found: C, 65.68; H, 9.14; N, 3.02.

20β-Dimethylamino-5α-pregnan-3β-yl β-D-Glucoside Hydrochloride (XII).—A solution of 940 mg. of 20β-dimethylamino-5α-pregnan-3β-ol[21] in dry chloroform was treated with 1.88 g. of acetobromoglucose in the usual way yielding a gum which crystallized on the addition of ethanol, 800 mg. m.p. 178–187°. This crude material was hydrolyzed in ethanol with sodium hydroxide and 450 mg. of product was obtained, m.p. 281–283°. The hydrochloride was prepared in the usual way, m.p. 260–261°.

Anal. Calcd. for $C_{29}H_{52}ClNO_6$: C, 63.77; H, 9.60; N, 2.56. Found: C, 63.14; H, 9.65; N, 2.67.

20β-Benzylideneamino-5-pregnen-3β-ol.—To a solution of 5.0 g. of 20β-amino-5-pregnen-3β-ol in 100 ml. of 2% ethanolic sodium hydroxide was added 4 ml. of freshly distilled benzaldehyde.[10] On short standing the product separated from solution. It was removed by filtration and after recrystallization from ethanol yielded 4.5 g. of product, m.p. 192–194°.

Anal. Calcd. for $C_{28}H_{39}NO$: C, 82.91; H, 9.69. Found: C, 82.85; H, 9.78.

20β-Benzylideneamino-5-pregnen-3β-ol 3,4,5-Trimethoxybenzoate.—A solution of 2.0 g. of the above N-benzylidene and 2.0 g. of 3,4,5-trimethoxybenzoyl chloride in 20 ml. of pyridine was allowed to stand about 60 hr. at room temperature. The excess acid chloride was decomposed by the gradual addition of ice and finally the reaction mixture was poured into water. The precipitated material was extracted with chloroform and the organic phase washed with dilute hydrochloric acid and water. After drying the solvent was removed and the crystalline residue was recrystallized from ethanol yielding 2.1 g. of material, m.p. 179–181°.

Anal. Calcd. for $C_{38}H_{49}NO_5$: C, 76.09; H, 8.24. Found: C, 75.89; H, 8.31.

20β-Amino-5-pregnen-3β-ol 3,4,5-Trimethoxybenzoate Hydrochloride (XV).—One g. of the above substance was dissolved in warm methanol and subjected to catalytic hydrogenolysis using 800 mg. of 10% palladium on charcoal at atmospheric pressure. When the theoretical quantity of hydrogen had been taken up the catalyst was removed and the solvent concentrated to dryness. The resulting amorphous material was dissolved in ethanol and one drop of concentrated hydrochloric acid was added. Ether was then dropped in until the solution became cloudy. On standing crystals formed which were removed by filtration and washed with ether, 245 mg., m.p. 296–297° (dec.).

Anal. Calcd. for $C_{31}H_{46}ClNO_5$: C, 67.90; H, 8.45. Found: C, 67.90; H, 8.64.

17β-Trifluoroacetamido-5-androsten-3β-ol.—A cold solution of 3.5 g. of 17β-amino-5-androsten-3β-ol[22] in 25 ml. of dry pyridine was treated with 8.0 g. of trifluoroacetic anhydride according to the directions previously described for the preparation of the 20-trifluoroamides. On working up the reaction 2.5 g. of crude 3,17-diacylated compound was obtained, m.p. 176–178°. This material was hydrolyzed as described for the preparation of II and yielded 1.6 g. of product, m.p. 219–222°, after recrystallization from methanol–water and from ethyl acetate–hexane.

Anal. Calcd. for $C_{21}H_{30}F_3NO_2$: C, 65.50; H, 7.79. Found: C, 65.77; H, 8.38.

17β-Trifluoroacetamido-5-androsten-3β-yl β-D-Glucoside Tetraacetate.—The glucoside acetate was prepared from 870 mg. of the above amide by the method already mentioned using 1.85 g. of acetobromglucose, 1.17 g. of silver oxide and 1.0 g. of Drierite in 50 ml. of chloroform. The product, 520 mg., was recrystallized from hexane, m.p. 206–207°.

Anal. Calcd. for $C_{35}H_{48}F_3NO_{11}$: C, 58.73; H, 6.75. Found: C, 58.34; H, 6.65.

17β-Amino-5-androsten-3β-yl β-D-Glucoside Hydrochloride (XVII).—A 400 mg. sample of the above glucoside

(18) F. J. Bates and Associates, "Polarimetry, Saccharimetry and the Sugars," National Bureau of Standards Circular C 440, Washington, D. C., 1942, p. 493.

(19) C. Huebner, R. Overman and K. Link, *J. Biol. Chem.*, **155**, 615 (1944); O. Touster and V. Reynolds, *ibid.*, **197**, 863 (1952).

(20) H. Bredereck and E. Hambsch, *Ber.*, **87**, 38 (1954).

(21) V. Cerný, L. Lábler and F. Šorm, *Coll. Czech.*, **22**, 86 (1957).

(22) L. Ruzicka and M. Goldberg, *Helv. Chim. Acta*, **19**, 107 (1936).

acetate was hydrolyzed with 5% alcoholic sodium hydroxide in the usual manner. The crude material was converted to the hydrochloride for purification. After recrystallization from ethanol–water there was obtained 250 mg. of hydrochloride, m.p. > 300° (dec.).

Anal. Calcd. for $C_{25}H_{42}ClNO_6$: C, 61.50; H, 8.68. Found: C, 61.28; H, 8.72.

A 200 mg. sample of the hydrochloride was dissolved in warm ethanol–water and basified with 20% sodium hydroxide solution. The precipitate was removed by filtration and washed well with water and with ethanol yielding 120 mg. of base, m.p. 276–278°.

Anal. Calcd. for $C_{25}H_{41}NO_6$: C, 66.49; H, 9.15. Found: C, 66.36; H, 9.12.

17β-Amino-5α-androstan-3β-yl β-D-Glucoside Hydrochloride (XVIII).—A solution of 488 mg. of the above hydrochloride in 35 ml. of acetic acid was hydrogenated at atmospheric pressure using 250 mg. of platinum oxide catalyst. The theoretical amount of hydrogen, 24 ml., was taken up within 30 minutes. The catalyst was removed by filtration and the solvent evaporated *in vacuo*. The residue was dissolved in ethanol–water and a few drops of hydrochloric acid added. On standing the salt precipitated and was recrystallized from ethanol–water, 350 mg., m.p. > 300° (dec.).

Anal. Calcd. for $C_{25}H_{44}ClNO_4$: C, 61.27; H, 9.05; N, 2.80. Found: C, 60.49; H, 8.98; N, 2.90.

17β-Amino-5α-androstan-3β-yl L-Arabinoside Hydrochloride (XX).—3β-Hydroxy-5α-androstan-17-one (7.7 g.) was reacted with 18 g. of acetobromarabinose according to the procedure described previously for III and yielded 9.0 g. of crude product. Recrystallization from ethanol–water gave 3.5 g. of pure 17-keto-5α-androstan-3β-yl L-arabinoside triacetate, m.p. 186°.

Anal. Calcd. for $C_{30}H_{44}O_9$: C, 65.67; H, 8.08. Found: C, 65.42; H, 7.96.

Conversion of the above product to the 17-oxime and hydrogenation of this material in acetic acid solution produced 2.5 g. of 17β-amino-5α-androstan-3β-yl L-arabinoside triacetate (XIX), m.p. 105–110°.

Anal. Calcd. for $C_{30}H_{47}NO_8$: C, 65.55; H, 8.62; N, 2.55. Found: C, 65.33; H, 8.74; N, 2.27.

Hydrolysis of 2.0 g. of the triacetate with barium methoxide in methanol[18] and conversion of the resulting base to the hydrochloride yielded 1.0 g. of final product, m.p. 235° (dec.). In spite of repeated purification attempts a satisfactory analytical sample could not be obtained.

Anal. Calcd. for $C_{24}H_{42}ClNO_5$: C, 62.65; H, 9.20; N, 3.04; Cl, 7.71. Found: C, 60.85; H, 9.08; N, 2.79; Cl, 8.06.

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF SYNTEX, S. A., MEXICO, D. F., MEX.]

Optical Rotatory Dispersion Studies. XXXVII.[1,2] Steroids. CXLVI.[3] On the Mechanism and Stereochemical Course of the Bromination of 3-Keto Steroids and their Enol Acetates

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Kinetically controlled bromination of 2α-methylandrostan-17β-ol-3-one acetate (VIII), androstan-17β-ol-3-one acetate (XIV), their 19-nor analogs (Xa, XIIa) and their respective enol acetates (IX, XI, XIII, XV) has led to the following conclusions: (a) in the presence of steric inhibition, the kinetic product is the equatorial and not the axial bromo ketone; (b) in the absence of such steric factors, appreciable amounts of equatorial bromo ketone may accompany the axial isomer. These results require some modification of Corey's (ref. 5) concept that the bromination product of kinetic control is always the axially oriented bromo ketone. Comparison experiments of ketones and their enol acetates indicate operation of a similar mechanism, which cannot involve diaxial opening of an intermediate bromonium ion since Br–Cl led to bromo- rather than chloro-ketones. Attention is called to the observation that the exclusive formation of Δ^2-enols of 3-keto steroids is altered upon removal of the angular methyl group, appreciable amounts of the Δ^3-enol being observed among 19-nor-3-keto steroids.

Introduction

In the two preceding papers[1,2] it was shown that kinetically controlled bromination of a 2α-methyl-3-keto-5α steroid I or its enol acetate leads to the 2α-bromo-2β-methyl-3-ketone II existing in the boat conformation, while the product of thermodynamic control is the 2β-bromo-2α-methyl-3-ketone III. This observation, which was first uncovered by optical rotatory dispersion measurements,[4] raises some interesting questions with respect to the stereochemical course and mechanism of the bromination of keto steroids and their enol acetates. The present investigation represents an experimental attempt to answer some of the outstanding problems in this field.

The most important and generally accepted views on the stereochemistry of the bromination of cyclohexanones in general and keto steroids in particular are due to Corey.[5] These generalizations state that the kinetically controlled bromination product is *always*[5b] that in which the bromine atom assumes an axial orientation, because orbital overlap in the transition state is most favorable in such a geometric arrangement. If there exist no serious steric interactions between the axial bromine atom and other substituents (*e.g.*, an axial methyl group two carbon atoms removed) then the kinetic product is also the thermodynamically favored one. In the presence of such steric interference, the axial bromo ketone is converted into the equatorial one under conditions of thermodynamic control. Application of these rules[5] to the bromination of 2α-methyl-3-keto steroids (I) leads to the conclusion[6] that the kinetic product should be the 2β-bromo-2α-methyl-3-ketone III which is contrary to the experimental observations.[1,3] A possible rationalization for this divergence would be the assumption that bromination of 2α-methyl-3-keto steroids pro-

(1) Paper XXXVI, C. Djerassi, N. Finch, R. C. Cookson and C. W. Bird, THIS JOURNAL, **82**, 5488 (1960).

(2) α-Haloketones (Part 8); for Part 7 see ref. 1

(3) Paper CXLV, R. Mauli, H. J. Ringold and C. Djerassi, THIS JOURNAL, **82**, 5494 (1960).

(4) C. Djerassi, "Optical Rotatory Dispersion. Applications to Organic Chemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1960.

(5) (a) E. J. Corey, THIS JOURNAL, **75**, 2301 (1953); **76**, 175 (1954); (b) *Experientia*, **9**, 329 (1953).

(6) See Y. Mazur and F. Sondheimer, THIS JOURNAL, **80**, 5220 (1958).