darkening above 198°, λ_{max} 240.5 mμ (16,000), $[\alpha]_D$ + 102°. A qualitative test for fluorine was negative.[8]

Anal. Calcd. for $C_{23}H_{29}ClO_5$: C, 65.65; H, 6.95; Cl, 8.43. Found: C, 65.99; H, 7.14; Cl, 8.38.

A final crystallization of the rod-like crystals from acetone gave 236 mg. of pure 9α-chloro-11β-fluoro-17α,21-dihydroxy-4-pregnene-3,20-dione 21-acetate, m.p. 276–277.5° dec., λ_{max} 238 mμ (17,800), $[\alpha]_D$ +149°. A positive Beilstein test and a positive qualitative fluorine test were obtained.

Anal. Calcd. for $C_{23}H_{30}ClFO_5$: C, 62.65; H, 6.86. Found: C, 62.47; H, 6.65.

9α-Bromo-11β-fluoro-4-pregnene-3,20-dione (II). To a solution of 1.00 g. of 4,9(11)-pregnadiene-3,20-dione[9] in 15 ml. of hydrogen fluoride–pyridine reagent was added 0.66 g. (1.5 equiv.) of *N*-bromoacetamide. After 30 min. at room temperature the reaction mixture was partitioned between 0.20 l. of ethyl acetate and 0.10 l. of water. The organic phase was washed with water and with saturated aqueous sodium bicarbonate solution. After being dried with anhydrous sodium sulfate, the solvent was removed by distillation at reduced pressure. The residue was crystallized from acetone–petroleum ether to give 205 mg. of crude product, m.p. 148–152° dec. Successive crystallizations from acetone and acetone–petroleum ether failed to give a product of constant melting point. The highest melting point was 165.5–172° dec. and the melting point of the analytical sample was 161–165° dec., λ_{max} 240 mμ (15,700). A positive Beilstein test and a positive qualitative fluorine test were obtained.

Anal. Calcd. for $C_{21}H_{28}BrFO_2$: C, 61.31; H, 6.86. Found: C, 59.59; H, 6.41.

An attempt was made to convert II to its bisethylene ketal using ethylene glycol, *p*-toluenesulfonic acid, and benzene in the usual way.[10] Chromatography of the product on silica gel failed to yield any product corresponding to the ketal, but a pure sample, m.p. 159–161° dec., of 9α-bromo-11β-fluoro-4-pregnene-3,20-dione was obtained by crystallization from acetone–petroleum ether of those fractions eluted from the column with 5% and with 10% ethyl acetate in benzene solutions.

Anal. Calcd. for $C_{21}H_{28}BrFO_2$: C, 61.31; H, 6.86. Found: C, 61.22; H, 6.49.

Preparation of 9α-bromo-11β-fluoro-4-pregnene-3,20-dione from 9α-bromo-11β-hydroxy-4-pregnene-3,20-dione. A solution of 0.50 g. of 9α-bromo-11β-hydroxy-4-pregnene-3,20-dione[11] in 10 ml. of hydrogen fluoride–pyridine reagent was kept at room temperature for 2 hr. The reaction mixture was partitioned between 0.10 l. of benzene and 0.10 l. of water. The benzene solution was washed with water and with saturated aqueous sodium bicarbonate solution. After drying with anhydrous sodium sulfate the solvent was removed by distillation at reduced pressure. Crystallization from acetone–petroleum ether and from benzene–petroleum ether gave 38 mg. of 9α-bromo-11β-fluoro-4-pregnene-3,20-dione, identical in melting point and infrared spectrum with II prepared from 4,9(11)-pregnadiene-3,20-dione. Admixture of the two samples did not depress the melting point.

9α-Chloro-11β-fluoro-4-pregnene-3,20-dione (III). Cold hydrogen fluoride–pyridine reagent (10 ml.) was added to a mixture of 1.00 g. of 4,9(11)-pregnadiene-3,20-dione and 426 mg. (1 equiv.) of *N*-chlorosuccinimide. After 1 hr. at 3° the crude product was isolated as described under the preparation of II. The crude product was triturated with ether and there was obtained 230 mg. of crystals, m.p. 135–165°. Repeated crystallization from ether–petroleum ether and from acetone–petroleum ether gave 60 mg. of pure 9α-chloro-11β-fluoro-4-pregnene-3,20-dione, m.p. 179–180°, λ_{max} 238 mμ (17,200). The analytical sample gave a positive Beilstein test and a positive qualitative fluorine test.

Anal. Calcd. for $C_{21}H_{28}ClFO_2$: C, 68.74; H, 7.69. Found: C, 68.77; H, 7.66.

9α-Bromo-11β-fluoro-17α-hydroxy-4-pregnene-3,20-dione 17-acetate (IV). A mixture of 336 mg. of 17α-hydroxy-4,9(11)-pregnadiene-3,20-dione 17-acetate[12] and 126 mg. (1 equiv.) of *N*-bromoacetamide was treated with 4 ml. of cold hydrogen fluoride–pyridine reagent. After 1 hr. at 2° the crude product was isolated as described under the preparation of II. Trituration with ether gave a first crop of crystals, 163 mg., m.p. 190–193° dec. and a second crop, 67 mg., m.p. 185–187° dec. The crops were combined and the mixture was crystallized from ether and from acetone–petroleum ether. The yield of pure 9α-bromo-11β-fluoro-17α-hydroxy-4-pregnene-3,20-dione 17-acetate was 95 mg. The melting point was variable, being 185–188° dec. and 193–196° dec., λ_{max} 240 mμ (17,100), $[\alpha]_D$ +85°. The analytical sample gave a positive Beilstein test and a positive qualitative test for fluorine.

Anal. Calcd. for $C_{23}H_{30}BrFO_4$: C, 58.85; H, 6.44. Found: C, 58.68; H, 6.31.

The attempted preparation of 9α,11β-difluoro-4-pregnene-3,20-dione. A solution of 100 mg. of 9α-fluoro-11β-hydroxy-4-pregnene-3,20-dione[11] in 10 ml. of hydrogen fluoride-pyridine reagent (77% hydrogen fluoride) was kept at room temperature overnight. The course of the reaction was followed by removing 2-ml. samples at 0.5, 1, 2, 4, and 18 hr. The samples were partitioned between ethyl acetate and water. The organic phase was washed with water, saturated aqueous sodium bicarbonate solution, and with water. After drying over anhydrous sodium sulfate the solvent was evaporated and the residue from each sample was submitted to infrared analysis. All of the samples gave a crystalline residue and all of the infrared spectra were identical with the spectrum of 9α-fluoro-11β-hydroxy-4-pregnene-3,20-dione.

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(9) C. W. Shoppee and T. Reichstein, *Helv. Chim. Acta*, **24**, 351 (1941).

(10) R. Antonucci, S. Bernstein, R. Littell, K. J. Sax, and J. R. Williams, *J. Org. Chem.*, **17**, 1341 (1952).

(11) Prepared according to the directions of J. Fried, J. E. Herz, E. F. Sabo, A. Borman, F. M. Singer, and P. Numerof, *J. Am. Chem. Soc.*, **77**, 1068 (1955).

(12) C. G. Bergstrom, R. T. Nicholson, R. L. Elton, and R. M. Dodson, *J. Am. Chem. Soc.*, **81**, 4432 (1959).

N-Acylation of 2-Amino-2-deoxy-D-glucose with Mixed Carboxylic Acid Anhydrides

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The acylation of amino compounds such as hydroxylamine,[1] substituted amino acids,[2] and amino acids[3] with mixed carboxylic acid anhydrides has been reported by several authors, and the

(1) T. Wieland and D. Stimming, *Ann.*, **579**, 97 (1953).

(2) T. Wieland and R. Sehring, *Ann.*, **569**, 122 (1950).

(3) J. R. Vaughan, Jr., and R. L. Osato, *J. Am. Chem. Soc.*, **73**, 5553 (1951); **74**, 676 (1952).

mechanism of the splitting of mixed acid anhydrides has also been well discussed.[3-5]

The solvent, steric, and induction effects were the main object of these investigations. Vaughan and Osato[3] prepared peptides by the reaction of mixed carboxylic acid anhydrides with aromatic amines and stated that under anhydrous conditions the combined action of an electronic and a steric effect was the cause of the preferential formation of the peptide bond, whereas under aqueous conditions a marked change of the ratio of the acylation products was observed. Emery and Gold[4] have also studied the solvent effect on the composition of the products obtained by interaction of mixed acid anhydrides and primary amines. The mechanism of the reaction of symmetrical anhydrides with amines has also been reported.[5,6]

Inouye, Onodera, Kitaoka, and Hirano[7] have reported the *N*-acylation of D-glucosamine (2-amino-2-deoxy-D-glucose) with symmetrical acid anhydrides in methanol, in which free D-glucosamine has been formed and treated with the anhydrides under supersaturation conditions. In the present paper the reaction of D-glucosamine in methanol with mixed carboxylic anhydrides to produce *N*-acylated D-glucosamine is reported.

Acetic benzoic anhydride was treated with D-glucosamine in methanol, from which *N*-acetyl-D-glucosamine was isolated in 69%. The reaction of acetic palmitic anhydride with D-glucosamine gave *N*-palmitoyl-D-glucosamine in 63% yield and that of caprylic palmitic anhydride also gave rise to *N*-palmitoyl-D-glucosamine in 79% yield. The reaction of benzoic myristic anhydride with D-glucosamine resulted in the isolation of *N*-myristoyl-D-glucosamine in 86% yield, and acetic butyric anhydride with D-glucosamine gave *N*-butyroyl-D-glucosamine in over 50% yield. The reaction of benzoic phthalylglycine anhydride with D-glucosamine gave rise to *N*-(phthalylglycyl)-D-glucosamine in 65% yield. The results are listed in Table I.

TABLE I

N-ACYLATED-D-GLUCOSAMINES

Mixed Anhydrides	Reaction Products	Yields (%)
Acetic benzoic	*N*-Acetyl-D-glucosamine	69
Acetic palmitic	*N*-Palmitoyl-D-glucosamine	63
Benzoic myristic	*N*-Myristoyl-D-glucosamine	86
Caprylic palmitic	*N*-Palmitoyl-D-glucosamine	79
Acetic butyric	*N*-Butyroyl-D-glucosamine	50
Benzoic phthalyl-glycine	*N*-(Phthalylglycyl)-D-glucosamine	65

(4) A. R. Emery and V. Gold, *J. Chem. Soc.*, 1443, 1447, 1455 (1950); V. Gold and E. G. Jefferson, *J. Chem. Soc.*, 1409, 1416 (1953); V. Gold, J. Hilton, and E. G. Jefferson, *J. Chem. Soc.*, 2756 (1954).

(5) D. B. Deney and M. A. Greenbaum, *J. Am. Chem. Soc.*, **78**, 877 (1956).

(6) E. Berliner and L. H. Altschul, *J. Am. Chem. Soc.*, **74**, 4110 (1950).

It is reasonable to assume that in a polar solvent like methanol the ionization of a mixed anhydride, RCO—O—OCR′ will probably proceed according to $R'CO^+ + RCO_2^-$ provided that $R'CO_2H$ is a weaker acid than RCO_2H. Therefore, it is more probable to find in the solution $R'CO^+$ instead of RCO^+ ions. The theory has been predicted by Emery and Gold on the basis of acylium ion theory. Accordingly, it is clear that acetic benzoic anhydride will produce *N*-acetyl-D-glucosamine. The reaction in the present paper was not studied in a quantitative way but preparatively, and the mechanism is not discussed here. There is a report on the reaction of mixed anhydrides with aniline,[8] which resulted in the isolation of acetanilide in a larger amount and of benzanilide in a smaller amount from the reaction mixture of acetic benzoic anhydride with aniline. Acetic butyric anhydride with aniline produced *N*-butrylaniline in a larger amount.

The tendency of the reaction is clearly the same as in these reports. The procedure developed here will constitute an alternate method for the preparation of *N*-acyl-D-glucosamine, and some examples described here show that this procedure could be applied in general to prepare *N*-(*N*′-substituted amino acid)-D-glucosamines.

EXPERIMENTAL[9]

Reaction of acetic benzoic anhydride with D-*glucosamine.* Acetic benzoic anhydride was prepared according to the procedure of Autenrieth[10] and Nef.[11]

Acetic benzoic anhydride (8 g.) was added to the methanolic solution of D-glucosamine, which had been prepared by treating D-glucosamine hydrochloride (10 g.) in methanol (70 ml.) with metallic sodium (1.0 g.). The reaction mixture was allowed to stand at room temperature for 1 hr. and then at ice-box temperature overnight, during which time crystals deposited; yield, 7.1 g. (69%). The crude crystals were recrystallized twice from water-methanol; yield, 6.5 g. (63%); m.p. 204°, $[\alpha]_D^{14}$ +41° (*c* 2, water); mixed melting point with the authentic sample of *N*-acetyl-D-glucosamine showed no depression.

The mother liquor from the crude *N*-acetyl-D-glucosamine was concentrated to yield 5.4 g. (94%) of benzoic acid.

Reaction of acetic palmitic, caprylic palmitic, benzoic myristic, and acetic butyric anhydrides with D-*glucosamine.* Acetic palmitic and caprylic palmitic anhydrides were prepared by the procedures of Ralston and Reck[12] and of Chiozza.[13]

A. *Acetic palmitic anhydride.* To anhydrous sodium acetate (1 mole) was added palmitoyl chloride (1.03 moles) and the reaction mixture was heated at 90° for 30 min. The product was recrystallized twice from petroleum ether (b.p. 40–60°), yield, 62%, m.p. 62–63°.

(7) Y. Inouye, K. Onodera, S. Kitaoka, and S. Hirano, *J. Am. Chem. Soc.*, **78**, 4722 (1956).

(8) C. D. Hurd and M. F. Dull, *J. Am. Chem. Soc.*, **54**, 3427 (1932).

(9) All melting points are uncorrected.

(10) W. Autenrieth, *Ber.*, **20**, 3189 (1887); **34**, 168 (1901).

(11) J. U. Nef, *Ann.*, **298**, 287 (1897).

(12) A. W. Ralston and R. A. K. Reck, *J. Org. Chem.*, **11**, 624 (1946).

(13) L. Chiozza, *Ann.*, **91**, 104 (1854).

Anal. Calcd. for $C_{18}H_{34}O_3$: C, 72.43; H, 11.48. Found: C, 72.96; H, 11.87.

B. *Caprylic palmitic anhydride.* To sodium palmitate (1 mole) which had been prepared from sodium methoxide and palmitic acid was added caproyl chloride (1.03 moles) and the reaction mixture was kept at 90° for 2 hr. The reaction product upon recrystallization twice from petroleum ether (b.p. 40–60°) gave caprylic palmitic anhydride in 85% yield, m.p. 58–59°.

Anal. Calcd. for $C_{24}H_{46}O_3$: C, 75.35; H, 12.12. Found: C, 75.71; H, 12.39.

C. *Benzoic myristic anhydride.* Benzoic myristic anhydride was prepared by the procedure reported by Ralston and Reck.[12]

D. *Acetic butyric anhydride.* Acetic butyric anhydride was obtained as a by-product in the preparation of the symmetrical anhydride,[10] b.p. 155–157°.

E. *The reactions with* D-*glucosamine.* To the methanolic solution of D-glucosamine was added an equivalent amount of the mixed acid anhydride, and the reaction mixtures were placed at ice-box temperature overnight. The crystals deposited were collected and recrystallized from ethanol. The reaction of acetic palmitic anhydride yielded *N*-palmitoyl-D-glucosamine (63%), m.p. 202–203°.

Anal. Calcd. for $C_{22}H_{44}O_6N$: C, 63.28; H, 10.38; N, 3.55. Found: C, 63.14; H, 10.42; N, 3.16.

The reaction of caprylic palmitic anhydride with D-glucosamine gave rise to *N*-palmitoyl-D-glucosamine in 79% yield, m.p. 201–202°.

Anal. Calcd. for $C_{22}H_{44}O_6N$: C, 63.28; H, 10.38; N, 3.55. Found: C, 63.24; H, 10.24; N, 3.40.

Benzoic myristic anhydride with D-glucosamine gave *N*-myristoyl-D-glucosamine in 86% yield, m.p. 208–209°, $[\alpha]_D^{16}$ +62° (*c* 1, water).

Anal. Calcd. for $C_{10}H_{19}O_6N$: C, 48.18; H, 7.68; N, 5.61. Found: C, 47.87; H, 7.60; N, 5.60.

Acetic butyric anhydride with D-glucosamine gave *N*-butyroyl-D-glucosamine in yields over 50%, m.p. 208–209°, $[\alpha]_D^{16}$ +62° (*c* 1, water).

Anal. Calcd. for $C_{10}H_{19}O_6N$: C, 48.18; H, 7.68; N, 5.61. Found: C, 47.87; H, 7.60; N, 5.60.

The reaction of benzoic phthalylglycine anhydride with D-*glucosamine.* Benzoic phthalylglycine anhydride was prepared by the procedure reported by Wieland, Kern, and Sehring.[14]

Benzoic phthalylglycine anhydride with D-glucosamine gave rise to *N*-(phthalylglycyl)-D-glucosamine in 65% yield, m.p. 218–219°, $[\alpha]_D^{18}$ +48° (*c* 1, water).

Anal. Calcd. for $C_{16}H_{18}O_8N_2 \cdot H_2O$: C, 50.00; H, 5.52; N, 7.29. Found: C, 49.83; H, 5.21; N, 7.36.

The water of crystallization was lost on drying for 1 hr. at 100° *in vacuo*.

Anal. Calcd. for $C_{16}H_{18}O_8N_2$: C, 52.46; H, 4.95; N, 7.65. Found: C, 51.91; H, 4.58; N, 7.83.

Refluxing phthalylglycine and acetic anhydride produced unstable acetyl phthalylglycine anhydride, which on reaction with D-glucosamine yielded *N*-(phthalylglycyl)-D-glucosamine.

*Tetra-O-acetyl-N-(phthalylglycyl)-*D-*glucosamine.* *N*-(Phthalylglycyl)-D-glucosamine (5 g.) was acetylated with the mixture of acetic anhydride (20 ml.) and pyridine (20 ml.). The acetylation product was treated in the usual manner. Recrystallization was effected from ethanol, yield, 4.0 g., m.p. 202–203°, $[\alpha]_D^{16}$ +29° (*c* 1, $CHCl_3$).

Anal. Calcd. for $C_{24}H_{26}O_{12}N_2$: C, 53.93; H, 4.90; N, 5.24. Found: C, 54.26; H, 5.19; N, 5.53.

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(14) T. Wieland, W. Kern, and R. Sehring, *Ann.*, **569**, 117 (1950).

Constituents of the Saguaro (*Carnegiea gigantea*). I. Proximate Analysis of the Woody Tissues

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This report describes the results of a proximate chemical analysis of the "woody" portions of the giant Saguaro cactus, *Carnegiea gigantea, Br. and R.* This initial investigation is part of a larger study of the Saguaro, which has been undertaken in this laboratory to establish its relationship to other xerophytic plants with respect to identity and mode of formation of polysaccharides, lignin, suberin, and extractives.

The Saguaro, largest of the United States cacti, is endemic to the Sonoran desert. It thrives under conditions of high temperature, low rainfall, and loose rocky soil. Individual plants may attain a height of fifty feet, a weight of six tons and an age of 200 years. The ability of the cactus to accumulate and retain water enables it to flower and bloom during periods of prolonged drought. The cortex of the plant is unusually large, permitting a variety of studies not possible with the smaller cortex of other dicotyledons.

A unique feature of the plant is the inner framework of ligniferous ribs, the secondary xylem, which is its main structural member. The chemical composition of this woody rib material as related to the composition of typical heartwoods is of interest to organic chemists and taxonomists alike. Another interesting feature of this plant is its response to injury or bacterial infection following injury.[1] A hard callus tissue is formed in concentric layers around the injured part. This callus tisse may extend deep into the pulpy cortex, sometimes more than six inches and, like the ribs, it is highly ligniferous. The mechanism of callus formation, as well as the formation of related wound tissues and the mechanism of abscission, have been studied in other plants from an anatomical, physiological, and histochemical viewpoint.[3,4] Bonner[6,7] and

(1) Alice M. Boyle, *Phytopathology*, **39** (12), 1029–52, (1949).

(2) (a) B. L. Browning and I. H. Isenberg, "Wood Chemistry," L. E. Wise and E. C. John, eds., Reinhold Publishing Company, New York, 1952, Chapt. 34. (b) B. L. Browning, *Wood Chemistry*, L. E. Wise and E. C. John, eds., Reinhold Publishing Company, New York, 1952, Chapt. 32.

(3) E. J. Butler and S. G. Jones, *Plant Pathology*. MacMillan Company, London, 1949.

(4) E. Gaumann, *Principles of Plant Infection*, Hafner Publishing Company, New York, 1950.

(5) L. E. Wise, M. Murphy, and A. A. D'Addieco, *Paper Trade J.*, **122**, #2, 35 (1946).

(6) J. Bonner, J. M. Henderson, and Mary E. Durrell, *Am. J. Botany*, **39**, 467–73 (1952).

(7) J. Bonner, *Plant Biochemistry*, Academic Press, New York, 1950.