[CONTRIBUTION FROM THE BIOCHEMICAL LABORATORY, COLLEGE OF AGRICULTURE, KYOTO UNIVERSITY]

Some Fatty Acid Derivatives of D-Glucosamine

BY YOSHIYUKI INOUYE, KONOSHIN ONODERA, SHOZABURO KITAOKA AND SHIGEHIRO HIRANO

RECEIVED APRIL 24, 1956

Some N-acyl and N-acyl-tetra-O-acyl-derivatives and some mixed acyl compounds of D-glucosamine (2-amino-2-deoxy-D-glucose) were prepared by the action of the acid anhydrides and acid chlorides. The acyl groups used were those of the even-numbered saturated fatty acids. The N-acyl-D-glucosamines prepared exhibited the identical absorption maxima in the Morgan and Elson reaction.

The isolation from biological materials of a compound composed of amino sugar and higher fatty acid has not been reported, but this group of amino sugar derivatives is of great interest because amino sugars are sometimes found in combination with fatty acids, *e.g.*, in the lipopolysaccharides of some microörganisms.[1] The present work was undertaken for the purpose of investigating the preparation and properties of some *N*-acyl and *N*-acyl-tetra-*O*-acyl derivatives of D-glucosamine (2-amino-2-deoxy-D-glucose), in which the acyl group was derived from even-numbered saturated fatty acids.

Some acyl derivatives of this amino sugar with lower fatty acids have been previously reported in the literature. Wholly and partially acetylated D-glucosamines have been extensively investigated,[2,3] and *N*-propionyl-D-glucosamine[4] and derivatives of *N*-formyl-, *N*-propionyl- and *N*-butyroyl-D-glucosamine[5] have been prepared. There is only one report, by Jones, Kaye and Stacey,[6] concerning the preparation of a higher fatty acid derivative of D-glucosamine, namely, *N*-stearoyl-tetra-*O*-stearoyl-D-glucosamine. Some fatty acid derivatives of D-glucose have been synthesized.[7]

The *N*-acylation and polyacylation of D-glucosamine were carried out with fatty acid anhydrides and chlorides. Treatment of D-glucosamine with the acid anhydrides in anhydrous methanol or with the acid chlorides in water (with alternate addition of sodium hydroxide) gave rise to the *N*-acylated D-glucosamines, and treatment of D-glucosamine with acid anhydrides in pyridine or with acid chlorides in a mixture of chloroform and pyridine produced the *N*-acyl-tetra-*O*-acyl-D-glucosamines. The *N*-acylation was most effectively carried out by the addition of the respective acid anhydrides to a supersaturated, methanolic solution of D-glucosamine. It is known that D-glucosamine is liberated from its salts by treatment with strong bases[8,9] and that it is slightly soluble in alcohol (1 part in 38 of boiling methanol[8]). However, it was found that a supersaturated solution of D-glucosamine is obtained when a suspension of D-glucosamine in an adequate volume of methanol is treated with an equivalent amount of sodium methoxide. The supersaturated, methanolic solution of D-glucosamine so formed can be maintained for some time at room temperature in concentrations as high as 10% or more, and upon the addition of a slight excess of an acid anhydride at room temperature an almost quantitative yield of quite pure *N*-acyl-D-glucosamine is precipitated. *N*-Benzoyl-D-glucosamine which was previously prepared[10] by the action of benzoyl chloride was also prepared by this method from benzoic anhydride.

This procedure offers a simplified method for the preparation of *N*-acetyl-D-glucosamine.[11] This important derivative of D-glucosamine has hitherto been prepared from D-glucosamine hydrochloride in methanol by the action of acetic anhydride and silver acetate[12]; or with acetic anhydride and anhydrous sodium acetate[13]; in *N*,*N*-dimethylformamide with acetic anhydride at low temperature,[4] and in aqueous methanol with acetic anhydride in the presence of Dowex-1.[14] The present method needs no catalyst and no particular treatment, but gives preparations of high purity in good yield. The crude *N*-acetyl-D-glucosamine that immediately starts to crystallize from the methanolic supersaturated solution of D-glucosamine on the addition of acetic anhydride with completion of crystallization overnight in the refrigerator has m.p. 200 to 204°, being sufficiently pure for ordinary purposes. One recrystallization raised the melting point to 208° (uncorrected).

The action of fatty acid chlorides on D-glucosamine resulted in smaller yields and lower purity of the obtained *N*-acylated-D-glucosamines than did the action of the acid anhydrides on D-glucosamine, and the chlorides of lower fatty acids gave variable yields of the corresponding *N*-acyl-D-glucosamines. Concerning the higher homologs, the preparations from the acid chlorides showed physical constants identical with those from the corresponding acid anhydrides.

N-Acyl-tetra-*O*-acyl-D-glucosamines were prepared by either one or both methods in which the acid anhydride or acid chloride was allowed to react with D-glucosamine, D-glucosamine hydrochloride or *N*-acyl-D-glucosamine. Preparations

(1) W. N. Haworth, P. W. Kent and M. Stacey, *J. Chem. Soc.*, 1220 (1948); M. Ikawa, J. B. Koepfli, S. G. Mudd and C. Niemann, THIS JOURNAL, **75**, 3439 (1953); *cf.* O. Westphal and O. Lüderitz, *Angew. Chem.*, **66**, 407 (1954).

(2) A. B. Foster and M. Stacey, *Advances in Carbohydrate Chem.*, **7**, 247 (1952).

(3) P. W. Kent and M. W. Whitehouse, "Biochemistry of the Aminosugars," Butterworths, London, 1955.

(4) R. Kuhn and F. Haber, *Chem. Ber.*, **86**, 722 (1953).

(5) A. Neuberger and R. V. Pitt Rivers, *Biochem. J.*, **33**, 1580 (1939).

(6) A. S. Jones, M. A. G. Kaye and M. Stacey, *J. Chem. Soc.*, 5016 (1952).

(7) K. Hess and E. Messmer, *Ber.*, **54**, 499 (1921).

(8) R. Breuer, *ibid.*, **31**, 2193 (1898).

(9) O. Westphal and H. Holzmann, *ibid.*, **75**, 1274 (1942).

(10) M. Bergmann and L. Zervas, *ibid.*, **64**, 975 (1931); **65**, 1201 (1932).

(11) A preliminary report: Y. Inouye, K. Onodera, S. Kitaoka and T. Kirii, *Bull. Inst. Chem. Research, Kyoto Univ.*, **33**, 270 (1955).

(12) T. White, *J. Chem. Soc.*, 428 (1940).

(13) W. R. Smithes, *Biochem. J.*, **53**, xxix (1953).

(14) S. Roseman and I. Ludowieg, THIS JOURNAL, **76**, 301 (1954).

TABLE I

FATTY ACID DERIVATIVES OF D-GLUCOSAMINE

Derivatives	Methods of prepn.[a]	State[b]	M.p., °C, (uncor.)	$[\alpha]D^c$ (with solvent)	Yields,[d] %	Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
N-Acyl												
N-Acetyl[e]	A	C	208	+40.5(W)	q.	$C_8H_{15}O_6N$	43.43	43.10	6.84	6.82	6.33	6.21
N-Butyroyl	A, B	C	212	+149 (P)	q.	$C_{10}H_{19}O_6N$	48.18	47.97	7.68	7.46	5.62	5.27
N-Caproyl	A	W	217–218	+114 (P)	q.	$C_{12}H_{23}O_6N$	51.97	52.15	8.36	8.21	5.05	4.71
N-Capryloyl	A, C	W	215	+129 (P)	q.	$C_{14}H_{27}O_6N$	55.06	55.17	8.91	8.83	4.59	4.40
N-Caprinoyl	A, C	W	212–213	+100 (P)	q.	$C_{16}H_{31}O_6N$	57.63	57.62	9.37	9.32	4.20	3.92
N-Lauroyl	A, C	W	208–209	+98 (P)	q.	$C_{18}H_{35}O_6N$	59.81	59.43	9.76	9.73	3.88	3.83
N-Myristoyl	A, C	W	198	+79 (P)	q.	$C_{20}H_{39}O_6N$	61.67	61.50	10.09	10.11	3.60	3.51
N-Palmitoyl	A, C	W	202–203	+79 (P)	q.	$C_{22}H_{43}O_6N$	63.28	63.56	10.38	10.36	3.35	3.06
N-Stearoyl	A, C	W	194–195	+78 (P)	q.	$C_{24}H_{47}O_6N$	64.68	65.10	10.63	10.80	3.14	2.90
Pentaacyl												
Pentaacetyl[f]	E, H	C	138–139	+92 (C)	..	$C_{18}H_{23}O_{10}N$	49.35	49.36	5.95	5.96	3.60	3.61
Pentabutyroyl	D, H	S		+30 (C)	71	$C_{28}H_{43}O_{10}N$					2.64	2.16
Pentacaproyl	D, G, I	S		+23 (C)	63	$C_{36}H_{63}O_{10}N$					2.09	2.38
Pentacapryloyl	G, I	S		+21 (C)	..	$C_{46}H_{83}O_{10}N$					1.73	1.63
Pentacaprinoyl	G, I	W	97–100	+9 (C)	23	$C_{56}H_{103}O_{10}N$	70.78	69.92	10.92	10.60	1.47	1.63
Pentalauroyl	G	W	97–98	+14 (C)	40	$C_{66}H_{123}O_{10}N$	72.68	72.82	11.37	11.53	1.28	1.17
Pentamyristoyl	F	W	82–83	+8 (C)	60	$C_{76}H_{143}O_{10}N$	74.18	74.20	11.71	11.68	1.14	1.25
Pentapalmitoyl	F, I	W	78	+8 (C)	74	$C_{86}H_{163}O_{10}N$	75.36	75.55	11.99	11.95	1.02	1.05
Pentastearoyl[g]	F	W	75–76	+9 (C)	66	$C_{96}H_{183}O_{10}N$	76.30	76.29	12.21	12.27	0.93	0.95
Mixed acyl												
N-Acetyl-tetra-*O*-caprinoyl	I	S		+17 (C)	..	$C_{48}H_{87}O_{19}N$					1.67	1.47
Tetra-*O*-acetyl-*N*-caprinoyl	H	S		+51 (C)	..	$C_{24}H_{39}O_{10}N$					2.79	2.39
N-Acetyl-tetra-*O*-lauroyl	I	W	84–86	+10 (C)	88	$C_{56}H_{103}O_{10}N$	70.76	70.65	10.92	9.91	1.47	1.36
N-Acetyl-tetra-*O*-palmitoyl	I	W	80–84	+16 (C)	92	$C_{72}H_{135}O_{10}N$	73.60	74.07	11.58	11.58		
N-Caprinoyl-tetra-*O*-palmitoyl	I	W	63–64	+14 (C)	90	$C_{80}H_{151}O_{10}N$	74.78	74.74	11.84	11.81	1.09	1.09
Tetra-*O*-caprinoyl-*N*-stearoyl	I	W	53–54	+12 (C)	..	$C_{64}H_{119}O_{10}N$	72.36	72.35	11.29	12.45	1.32	1.28

[a] A, action of acid anhydride on D-glucosamine in MeOH; B, acetic butyric anhydride on D-glucosamine in MeOH; C, acid chloride and NaOH on D-glucosamine in water; D, acid anhydride on D-glucosamine in pyridine; E, acid anhydride on D-glucosamine hydrochloride in pyridine; F, acid chloride on D-glucosamine hydrochloride in pyridine and chloroform; G, acid chloride on D-glucosamine in pyridine and chloroform; H, acid anhydride on *N*-acyl-D-glucosamine in pyridine; I, acid chloride on *N*-acyl-D-glucosamine in pyridine and chloroform. [b] *C*, crystalline; *S*, sirup; *W*, waxy-crystalline. [c] Unless specified for mutarotation, the values are for those after standing for 24 hr.; solvent: W, water; P, pyridine; C, chloroform. [d] q. stands for almost quantitative and the yields of fully acylated derivatives are those after one or two recrystallizations. [e] Roseman and Ludowieg[14] report m.p. 210° (corrected), $[\alpha]^{28}D$ +41.0°. [f] Westphal and Holzmann[9] report for *N*-acetyl-tetra-*O*-acetyl-α-D-glucosamine m.p. 139°, $[\alpha]^{20}D$ +92°. [g] Jones, Kaye and Stacey[6] report m.p. 71–72°, $[\alpha]^{25}D$ +16°.

of *N*-acyl-tetra-*O*-acyl derivatives of D-glucosamine containing higher fatty acids were carried out not by the acid anhydride method but by the acid chloride method, and those containing lower fatty acids were not obtained by the acid chloride method but by the acid anhydride method. Some mixed acyl derivatives of D-glucosamine were prepared by the reaction of *N*-acyl-D-glucosamine with acylating agents of different fatty acids.

The fatty acid derivatives of D-glucosamine thus prepared are given in Table I with their physical properties. As shown in the Table I, *N*-acetyl- and *N*-butyroyl-, and *N*-acetyl-tetra-*O*-acetyl-D-glucosamines were crystalline, and other *N*-acylated derivatives and pentaacyl and mixed acyl compounds containing higher fatty acids were waxy crystals. Those derivatives containing lower members of higher fatty acids (C_6–C_{10}) were sirups. As may be seen from Table II, the solubilities of the substances are related to their physical state.

TABLE II

SOLUBILITY PROPERTIES OF FATTY ACID DERIVATIVES OF D-GLUCOSAMINE

State of derivatives	Water	Methanol	Hot ethanol
Crystalline	+[a]	±	+[b]
Sirup	−	+	+
Waxy-crystalline	−	−	+[c]

[a] *N*-Acetyl-tetra-*O*-acetyl-D-glucosamine, particularly the β-isomer, is slightly soluble. [b] *N*-Acetyl-D-glucosamine is sparingly soluble. [c] The solubility decreases with the increase in carbon atoms of the acyl group for *N*-acyl derivatives.

The reaction of a mixed acid anhydride, acetic butyric anhydride, with D-glucosamine in methanol produced *N*-butyroyl-D-glucosamine but not *N*-acetyl-D-glucosamine. A similar result has been obtained in preparing *N*-(*N*,*N*-phthaloyl-glycyl)-D-glucosamine by the action of the mixed acid anhydride, which was prepared from *N*,*N*-phthaloyl-glycine and acetic acid, on D-glucosamine in methanol.[15]

A widely used color reaction of the *N*-acyl amino sugars is the Morgan and Elson reaction.[16] Although the mechanism of this reaction is still not clear, formation of some kind of ring structure by the action of alkali has been postulated,[3] and some *N*-acyl-D-glucosamines examined have been found to be positive to this reaction.[3] The *N*-acyl (fatty acid) derivatives of D-glucosamine and *N*-benzoyl-D-glucosamine here prepared were submitted to this color reaction, and it was found that all of them showed quite similar absorption curves, with three absorption maxima at 510, 545 and 585 mμ, as previously found for *N*-acetyl-D-glucosamine.[17]

Experimental

D-Glucosamine Hydrochloride.—This material was prepared from crab shells after the method of Purchase and Braun.[18]

(15) Y. Inouye, K. Onodera, S. Kitaoka and S. Hirano, unpublished.

(16) W. T. J. Morgan and L. A. Elson, *Biochem. J.*, **28**, 988 (1934).

(17) Y. Inouye, K. Onodera and S. Kitaoka, *J. Agr. Chem. Soc. Japan*, **29**, 139 (1955); *Bull. Inst. Chem. Research, Kyoto Univ.* **33**, 215 (1955).

(18) E. R. Purchase and C. E. Braun, *Org. Syntheses*, **26**, 36 (1946).

1. Preparation of *N*-Acyl-D-glucosamines. A. With Acid Anhydrides.—The starting anhydrides of butyric acid and caproic acid, as well as acetic butyric anhydride were prepared by the method of Autenrieth.[19] The anhydrides of caprylic, capric, lauric, myristic, palmitic and stearic acids were prepared according to Holde and Gentner.[20]

(a) **General Procedure.**—D-Glucosamine hydrochloride was placed in 8–10 volumes of anhydrous methanol in which an equivalent amount of sodium had been dissolved. Upon gentle swirling, sodium chloride separated and was removed by filtration and washed with a small volume of methanol. An amount of acid anhydride from 1.2 to 1.5 equivalents was added gradually under mechanical stirring to the supersaturated solution of D-glucosamine at room temperature. In the case of the anhydrides of the fatty acids higher than C_{14} the addition was carried out at higher temperatures. Stirring was continued for some time, and then the reaction mixture was allowed to stand at room temperature. Crystallization usually ensued immediately and was completed by standing overnight at ice-box temperatures. The crude *N*-acyl-D-glucosamines were removed by filtration, washed with cold methanol and then repeatedly with ether to remove fatty acids, and dried at room temperature over concentrated sulfuric acid. The yields were nearly quantitative under adequate conditions. Recrystallization was usually effected from hot ethanol.

(b) ***N*-Acetyl-D-glucosamine.**—By the above general procedure, the crude *N*-acetyl-D-glucosamine was obtained in nearly quantitative yield, after overnight standing, with the m.p. ranging from 200 to 204°. Recrystallization was effected from the minimal quantity of water by the addition of ethanol and then ether to incipient turbidity to give colorless needles.

(c) ***N*-Benzoyl-D-glucosamine.**—The above procedure was applied to prepare *N*-benzoyl-D-glucosamine by using benzoic anhydride. After keeping the reaction mixture for 5 days in an ice-box, it was concentrated to dryness under reduced pressure and was recrystallized from hot methanol; m.p. 204–206° (uncor.), $[\alpha]^{16}D$ +31° (after 24 hr., *c* 2, water); Bergmann and Zervas[10] reported m.p. 196–200°.

Anal. Calcd. for $C_{13}H_{17}O_6N$: C, 55.12; H, 6.05; N, 4.95. Found: C, 54.62; H, 5.90; N, 4.88.

(d) ***N*-Butyroyl-D-glucosamine by the Acetic Butyric Anhydride Method.**—According to the above general procedure, acetic butyric anhydride was added to the methanolic solution of D-glucosamine. The *N*-butyroyl-D-glucosamine after recrystallization from ethanol melted at 208–209°, $[\alpha]^{16}D$ +62° (after 24 hr., *c* 1, water); mixed m.p. with the *N*-butyroyl-D-glucosamine prepared with butyric anhydride showed no depression.

Anal. Calcd. for $C_{10}H_{19}O_6N$: C, 48.18; H, 7.68; N, 5.62. Found: C, 47.87; H, 7.60; N, 5.60.

B. With Acid Chlorides.—The fatty acid chlorides were prepared according to Ralston and Selby.[21] D-Glucosamine hydrochloride (0.05 mole) was dissolved in 50 ml. of *N* NaOH. To this, under vigorous stirring at −10 to −15°, 0.06 mole of acid chloride and 51 ml. of *N* NaOH were added alternately in small portions. After leaving for 2 hr., the reaction product was filtered and washed with water until the filtrate no longer gave a positive reaction for chloride ion. Removal of fatty acid was effected by repeated washing of the product with ethanol and then ether. The residue was twice recrystallized from hot ethanol. The *N*-acyl-D-glucosamines (C_8 to C_{18}) prepared by acid chlorides showed respectively no depression in m.p. on admixture with the corresponding *N*-acyl-D-glucosamines prepared with acid anhydrides.

2. Preparation of *N*-Acyl-tetra-*O*-acyl-D-glucosamines. A. With Acid Anhydrides.—In 30 ml. of pyridine, 0.02 mole of D-glucosamine (prepared by adding ether to a supersaturated, methanolic solution of D-glucosamine, filtering and drying) was suspended. To this, 0.14 mole of acid anhydride was added and the reaction mixture was heated until the D-glucosamine went into solution. After cooling, it was allowed to stand for 3 days at room temperature, poured into ice-water, and extracted three times with chloroform. The extract was washed three times with *N* hydrochloric acid, twice with water, three times with *N* sodium hydroxide, and finally twice with water and dried over calcium chloride. *N*-Butyroyl-tetra-*O*-butyroyl- and *N*-capryloyl-tetra-*O*-capryloyl-D-glucosamine were obtained as sirups, after removing solvent, taking up in hot alcohol, treating with active carbon, evaporating under reduced pressure and drying in a desiccator over concentrated sulfuric acid. With acetic anhydride, this procedure yielded *N*-acetyl-tetra-*O*-acetyl-α-D-glucosamine.

B. With Acid Chlorides.—*N*-Myristoyl-tetra-*O*-myristoyl-, *N*-palmitoyl-tetra-*O*-palmitoyl- and *N*-stearoyl-tetra-*O*-stearoyl-D-glucosamine were prepared by the action of the corresponding acid chlorides on D-glucosamine hydrochloride. In a mixture of 30 ml. of dry chloroform and 10 ml. of dry pyridine, 0.01 mole of D-glucosamine hydrochloride was suspended. Ten ml. of dry chloroform containing 0.055 mole of the acid chloride was slowly added to this suspension at −10° under vigorous stirring. The crystalline addition compound of the acid chloride and pyridine appeared. The reaction mixture was maintained for some time at this temperature, brought to room temperature gradually and heated on a water-bath for 8 hr. with exclusion of moisture. After cooling, it was poured into ice-water and the resulting emulsion was extracted three times with chloroform. The extract was washed twice with water, twice with *N* hydrochloric acid, and finally twice with water and dried over calcium chloride. The dried extract was evaporated under reduced pressure to dryness and the residue was repeatedly washed with hot methanol to remove the free fatty acid. Crystallization was effected from a mixture of petroleum ether and ethanol (2:1) and recrystallization from hot ethanol.

The *N*-capryloyl-tetra-*O*-capryloyl-, *N*-caproyl-tetra-*O*-caproyl-, *N*-caprinoyl-tetra-*O*-caprinoyl and *N*-lauroyl-tetra-*O*-lauroyl derivatives were prepared by the reaction of the corresponding acid chlorides with D-glucosamine. To a suspension of 0.03 mole of D-glucosamine in a mixture of 60 ml. of dry chloroform and 50 ml. of dry pyridine, 0.16 mole of the acid chloride was added. The reaction mixture was poured into ice-water, and the chloroform extract of the reaction products was washed thoroughly with water and *N* hydrochloric acid. Removal of fatty acid was effected by treatment with *N* NaOH for pentacapryloyl- and pentacaproyl-D-glucosamines, and by treatment with hot methanol for pentacaprinoyl- and pentalauroyl-D-glucosamines.

3. Acylation of *N*-Acyl-D-glucosamines.—By the procedure described above with acid anhydrides, α-*N*-acetyl-tetra-*O*-acetyl-α-D-glucosamine and sirupy *N*-butyroyl-tetra-*O*-butyroyl-D-glucosamine were prepared from *N*-acetyl-D-glucosamine and *N*-butyroyl-D-glucosamine, respectively. The *N*-acetyl-tetra-*O*-acetyl-α-D-glucosamine showed no depression in m.p. on admixture with that prepared by the action of acetic anhydride on D-glucosamine hydrochloride.

Tetra-*O*-acetyl-*N*-caprinoyl-D-glucosamine was prepared by the action of acetic anhydride upon *N*-caprinoyl-D-glucosamine in a similar way. *N*-Caproyl-tetra-*O*-caproyl-, *N*-capryloyl-tetra-*O*-capryloyl- and *N*-caprinoyl-tetra-*O*-caprinoyl-D-glucosamine, all again sirupy, and *N*-palmitoyl-tetra-*O*-palmitoyl-D-glucosamine were prepared by the action of acid chlorides on *N*-acyl-D-glucosamines in chloroform–pyridine mixture. The following mixed acyl derivatives of D-glucosamine were prepared by the same method: *N*-acetyl-tetra-*O*-lauroyl-, *N*-acetyl-tetra-*O*-palmitoyl-, *N*-caprinoyl-tetra-*O*-palmitoyl- and tetra-*O*-caprinoyl-*N*-stearoyl.

4. The Morgan and Elson Reaction of *N*-Acyl-D-glucosamines.—The conditions of this color reaction were followed as described by Morgan and Elson.[16] Measurements of the absorption curves were made, from 15 min. to 1 hr. after the reaction, by means of a Beckman spectrophotometer (DU model).

KITASHIRAKAWA, KYOTO, JAPAN

(19) W. Autenrieth, *Ber.*, **34**, 168 (1901).

(20) D. Holde and R. Gentner, *ibid.*, **58**, 1418 (1925).

(21) W. Ralston and W. M. Selby, THIS JOURNAL, **61**, 1019 (1939).