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Note

Preparation of some novel *N,O*-acylchitosans

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N-Acylation and partial *O*-acylation occur in the reaction of chitosan with carboxylic anhydrides in aqueous acetic acid solutions[1] to afford *N,O*-acylchitosan gels[2]. *N,O*-Acylchitosans are essential as reference compounds for the study of the structural influence of various *O*-acyl groups upon the gel formation and of the *O*-acylation in gel media. *O*-Acetylchitin[1,3,4] and *N,O*-lauroylchitosan[5] have previously been reported. We now report the preparation of a series of homogeneously or heterogeneously *N,O*-acylated chitosans by the acylation of *N*-acylchitosans[6] with acid chlorides in pyridine.

As shown in Table I, *N,O*-acylchitosans were isolated in yields of 43–98% on the basis of complete *N,O*-acylation. All the products were insoluble in the common solvents examined: formic acid, 50% resorcinol, dimethyl sulfoxide, chloroform, and formamide. However, *N,O*-propionylchitosan was slightly soluble in formic acid and 50% resorcinol, and depolymerized products were soluble in chloroform (see footnote to Table I).

The presence of *N*- and *O*-acyl groups in the products was detected by i.r. absorptions at 1730 (C=O in *O*-acyl), 1650, and 1550 cm^{-1} (C=O and NH in *N*-acyl), and by weak or almost no i.r. absorption at ~3470 cm^{-1} (OH).

The degree of substitution (d.s.) for *O*-acyl groups in the products varied from 0.69 to 2.00. D.s. values of 1.44–2.00 were found in the *O*-acylated products of *N*-myristoyl-, *N*-palmitoyl-, and *N*-stearoyl-chitosans, and d.s. values of 0.69–1.16 in the *O*-acylated products of *N*-propionyl-, *N*-butyryl-, and *N*-hexanoyl-chitosans. The d.s. values for *O*-acyl groups are almost proportional to the number of carbon atoms of the *N*-fatty acyl groups. *N*-Acylchitosans having the higher-molecular-weight fatty acyl groups at C-2 of 2-acylamino-2-deoxy-D-glucoside residues may form molecular conformations in which free hydroxyl groups have less steric hindrance. Actually, chitosan hydroacetate has no steric hindrance for *N*-acylation with carboxylic anhydrides[6]. The d.s. values calculated from the g.l.c. data agree with those calculated from the nitrogen content (Table I).

TABLE I

PREPARATION AND PROPERTIES OF *N,O*-ACYLCHITOSANS

N,O-*Acylchitosan*	*Preparation method*[a]	*Yield* (%)	*D.s. for O-acyl group*[b]	
			From g.l.c. data	*From nitrogen value*[c]
N,O-Propionyl	A(3)	57		0.89
N,O-Butyryl	A(5)	63		1.00
N,O-Hexanoyl[d]	A(8)	43		1.01
N,O-Octanoyl	A(6)	75		1.09
N,O-Lauroyl	A(6)	98		1.89
N,O-Stearoyl	A(6)	98		1.95
N-Propionyl-*O*-palmitoyl	B	88	0.78	0.69
N-Propionyl-*O*-stearoyl	B	50	0.69	
N-Butyryl-*O*-lauroyl	B	56	0.92	0.86
N-Butyryl-*O*-palmitoyl	B	58	1.16	
N-Hexanoyl-*O*-palmitoyl	B	63	1.11	1.09
N-Hexanoyl-*O*-stearoyl	B	67	1.07	
N-Octanoyl-*O*-lauroyl	B	72	1.22	1.15
N-Octanoyl-*O*-palmitoyl	B	59	1.16	
N-Octanoyl-*O*-stearoyl	B	86	1.64	
N-Decanoyl-*O*-butyryl	C	82	1.05	1.00
N-Decanoyl-*O*-stearoyl	B	57	0.79	
N-Lauroyl-*O*-palmitoyl	B	66	1.22	1.19
N-Lauroyl-*O*-stearoyl	B	74	1.35	
N-Myristoyl-*O*-lauroyl	B	78	1.70	1.66
N-Myristoyl-*O*-palmitoyl	B	67	1.44	
N-Myristoyl-*O*-stearoyl	B	69	1.26	
N-Palmitoyl-*O*-lauroyl	B	98	2.00	1.98
N-Palmitoyl-*O*-stearoyl	B	80	1.81	
N-Stearoyl-*O*-lauroyl	B	98	2.00	
N-Stearoyl-*O*-palmitoyl	B	96	1.90	1.85
N-Benzoyl-*O*-lauroyl	B	82	1.43	
N-Benzoyl-*O*-palmitoyl	B	80	1.78	1.71

[a]See Experimental part in the text. The time (h) under reflux is shown in parentheses for procedure A. [b]D.s. values were calculated from the g.l.c. data and the nitrogen contents. [c]Calc. for $[C_6H_{10}NO_4(N\text{-Acyl})_{1.00}(O\text{-Acyl})_x(H)_y]_n$ in which $x + y = 2.00$ and x indicates the d.s. value for *O*-acyl group. [d]*N*-Hexanoylchitosan was heated under reflux with hexanoyl chloride in pyridine for 8 h. Depolymerized products ($R_{hexanoic\ acid}$ 0.39, 0.20, 0.10, and 0.03) were detected in the chloroform extract by t.l.c. in 85:15:2:3 (v/v) heptanol–ether–acetic acid–methanol.

EXPERIMENTAL

General. — *N*-Acylchitosans were prepared by the procedure previously described[6]. The g.l.c. analysis was performed at 60–230° with a Shimadzu GC-5A apparatus equipped with a hydrogen-flame ionization detector, with nitrogen as carrier gas, and with a glass column (2 m × 0.4 cm) packed with silicone OV-17 (5%) on Celite 545 (80–100 mesh). References to the other analytical methods and materials have been cited previously[6].

O-Acylation of N-*acylchitosans.* — Each of the dried *N*-acylchitosans (100 mg)

was swelled in pyridine (15 ml) by being kept overnight at room temperature. To the mixture was added 4 molar equiv. of the corresponding acid chloride per 2-acylamino-2-deoxy-D-glucoside residue under vigorous stirring at -10 to $-15°$. The mixture was heated for 3–8 h under reflux (procedure A), or for 24 h at 50° (procedure C) and then for 2 h under reflux (procedure B). After being cooled to room temperature, the reaction mixture was poured into acetone ($\sim$100 ml), and the mixture was stirred for 30 min at room temperature to afford a precipitate. The precipitate was filtered off, washed successively with acetone and ether, and dried over P_2O_5 for 5 h at 100° *in vacuo*.

Analysis of the d.s. values for O-acyl groups. — (*a*). *N,O*-Acylchitosan (20 mg each), in which the *N*- and *O*-acyl groups were different, was hydrolyzed in a sealed tube with 6M H_2SO_4 (1 ml) for 3 h at 100°. After being cooled, the sealed tube was opened, and the free fatty acids produced were extracted with ether (2 × 2 ml). The combined ether extracts were dried (Na_2SO_4), and methanol (2 ml) containing 1% *p*-toluenesulfonic acid was added. The mixture was heated under reflux for 1 h to afford the methyl esters of the fatty acids. An aliquot of the solution was analyzed by g.l.c. The d.s. values for the *O*-acyl group were calculated from the peak ratio of the *O*-fatty acyl group to the *N*-fatty acyl group which has the d.s. value[6] of 1.00.

(*b*) The d.s. values for *O*-acyl groups were calculated from the nitrogen contents.

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