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Synthesis, characterization, and reverse-micellar studies of some *N*-substituted derivatives of 6-amino-6-deoxy-1,2-*O*-isopropylidene-D-glucose

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Abstract

Dry heating of *N*-substituted hexadecylamines with 5,6-anhydro-1,2-*O*-isopropylidene-α-D-glucofuranose afforded glucose-based nonionic surfactants having a tertiary amino group linked to C-6 of the glucose moiety. These surfactants were tested for the solubilization of the L-amino acids tryptophan, tyrosine, phenylalanine, and cysteine in *n*-hexane. Reverse-micellar measurements revealed that (6,6′-hexadecylimino)bis(6-deoxy-1,2-*O*-isopropylidene)-α-D-glucofuranose is the most effective surfactant.

Keywords: *N*-substituted hexadecylamines; 5,6-Anhydro-1,2-*O*-isopropylidene-α-D-glucofuranose; Nonionic surfactants

1. Introduction

Apart from their large-scale use as cleansing agents, surfactants, both natural and synthetic, have widespread applications in biochemical research for solubilizing lipophilic components of various tissues and cellular structures, particularly membrane components [1]. For membrane solubilization nonionic surfactants are preferred over ionic surfactants because they do not denature proteins, are active over a wide pH range, and are less sensitive to multivalent cations and temperature. Most carbohydrate-based surfactants consist of disaccharide [2–6] and monosaccharide fatty acid esters [7–14]. Amino sugar-based surfactants, in which the hemiacetal linkage is protected, are more

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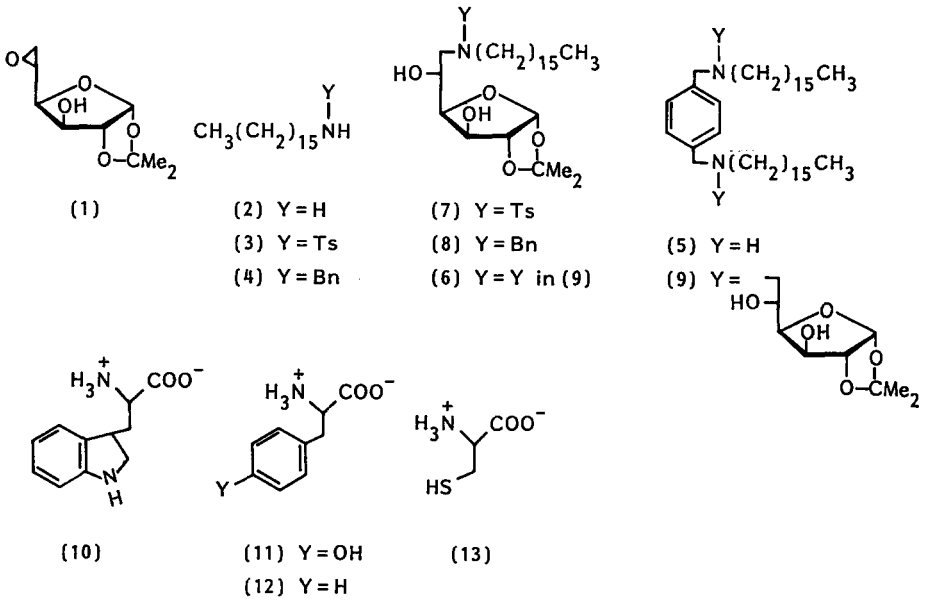

resistant to hydrolysis under alkaline conditions, but have not been studied intensively thus far.

This paper describes the synthesis of some new *N*-substituted 6-amino-6-deoxy-D-glucose derivatives as nonionic surfactants and their use as reverse micelles for the solubilization of L-amino acids in *n*-hexane. To our knowledge, this is the first example of using carbohydrate-based nonionic surfactants for reverse micellar studies.

2. Results and discussion

Synthesis and characterization. — The hexadecylamino sugars (**6–9**) were prepared by dry heating of the appropriate amine (**2–5**) and 5,6-anhydro-1,2-*O*-isopropylidene-α-D-glucofuranose (**1**) [15]. They possess a tertiary amino group linked to C-6 of the glucose moiety and their reducing function blocked in a cyclic acetal group. Opening of the epoxide ring of **1** by the various amines is expected [16] to proceed by exclusive nucleophilic attack on C-6 and without inversion at C-5.

The NMR signal assignments were based on previous studies by Abraham et al. [17] (for ^{1}H NMR) and Vyas et al. [18] (for ^{13}C NMR) on *O*-isopropylidene-D-hexoses. The chemical shifts (ppm) for H-1 (5.93 ± 0.5, doublet), H-2 (4.50 ± 0.1, doublet, H-3 (4.27 ± 0.4, doublet) and H-5 (4.0 ± 0.1, singlet) were constant and characteristic of these hydrogens. The signal of H-4 was also found constant, except in those cases where exact location could not be assigned because of overlapping signals. The most prominent shift was found in the H-6 signals. The methyl signal of the hexadecyl moiety was found

Table 1.
Critical micelle concentration (cmc) of the surfactant in mol L^{-1}

Surfactant	cmc
6	7.14×10^{-4}
7	8.33×10^{-4}
8	9.46×10^{-4}
9	2.30×10^{-4}

at 0.88 and its methylene signals at 1.22–1.30 merged with sugar isopropylidene signals. The position of methylene directly attached to nitrogen could not be assigned because of overlapping sugar signals.

^{13}C NMR signal assignments of all the carbons in each entire molecule were supported by off-resonance as well as by INEPT data. In sugar epoxide **1** and the amino derivatives **6–9**, the signals of the anomeric carbon, 1,3-dioxolane acetal carbon and methyl groups of the isopropylidene moiety were invariant at (105.2 ± 0.4), (111.6 ± 0.5), and (26.1 ± 0.3), respectively. The chemical shifts for C-2 (85.1 ± 0.2) and C-3 (75.0 ± 0.5) were also found nearly identical in all of the amino derivatives. The C-5 and C-6 signals are downfield as compared to those in **1**. The methyl signal of the hexadecyl moiety was found at 14.2 ppm and methylene carbons (except the one directly attached to nitrogen) at 22.6–31.8 ppm as multiplets.

Solubilization of amino acids. — Reverse micelle formation was studied by solubilization of the L-amino acids tryptophan, tyrosine, cysteine, and phenylalanine in *n*-hexane, using UV monitoring. The critical micelle concentrations (cmc) of the surfactants **6–9** (Table 1) were determined by the method of Gratzer and Beaven [19]. Keeping the concentration of surfactant well above its cmc, Table 2 shows that the micellar ratio was maximum in the case of phenylalanine and least in the case of cysteine. The amino acids were found to be solubilized in the order phenylalanine > tyrosine > tryptophan > cysteine, suggesting better incorporation of the former in the micellar phases.

Surfactant **6** gave the best results for the solubilization of different L-amino acids in apolar media, followed by **7**, **8** and **9**. This may be attributed to the more-hydrophilic character of **6**, that is, the additional hydroxyl groups in **6** facilitate the solubilization of amino acids via hydrogen bonding with the amino acids. In addition Table 2 shows that

Table 2.
Solubilization of amino acids in *n*-hexane with the help of micelles formed by nonionic surfactants

Surfactant	Micellar ratio Amino acid : molecules of micelle			
	Tryptophan	Tyrosine	Phenylalanine	Cysteine
6	1:59	1:44	1:14	1:239
7	1:250	1:91	1:27	1:289
8	1:122	1:139	1:40	1:267
9	1:227	1:150	1:14	1:211

a greater hydrophobic surface in a particular amino acid facilitates solubilization. Cysteine (**13**) has three polar hydrogen-bonding groups, namely SH, H_3N^+, and CO_2^- and no significant hydrophobic component. Tryptophan (**10**) and tyrosine (**11**) both also have three polar groups, namely NH, H_3N^+, and CO_2^- in the former and OH, H_3N^+, and CO_2^- in the latter, but also possess an aromatic ring that provides a flat hydrophobic surface in each. In phenylalanine (**12**) there are only two hydrophilic sites, H_3N^+ and CO_2^-, and a phenethyl hydrophobic site. This high hydrophobic–hydrophilic balance in **12** results in a greater solubilization behaviour. The efficiency of surfactant **6** also reveals that π–π interactions between surfactant and substrate do not exert any significant role in the solubilization.

Experimental

General methods.—Melting points were determined in capillaries and are uncorrected. ^{1}H NMR spectra were taken at 200 MHz with a Bruker FT NMR 200 spectrometer and at 60 MHz with a JEOL JNM PMX 60 SI spectrometer and ^{13}C NMR spectra at 200 MHz with a Bruker FT NMR 200 spectrometer. Tetramethylsilane was used as the internal reference for solutions in deuteriochloroform, unless otherwise stated and J values are given in Hz. IR spectra were determined with a SP3-300 Pye Unicam infrared spectrometer. UV spectra were recorded with a Shimadzu UV-160 UV-vis spectrophotometer. Optical rotations were measured on a Jasco DIP-360 digital polarimeter in a 1-dm cell. Elemental analysis was carried out on with a Perkin–Elmer 2400 instrument. Mass spectra were carried out at CDRI, Lucknow. Column chromatography was performed on silica gel (60–120 mesh) and TLC on plates coated with silica gel G. The spots were developed in iodine and/or by charring with 1% H_2SO_4 in water. Spectral grade *n*-hexane and doubly-distilled water were used for optical spectroscopy. Other reagents were used as obtained.

Solubilization of amino acids in apolar solvents.—The surfactant (5 mmol) in *n*-hexane (10 mL) was shaken with the amino acid (50 mg) for 20 min and filtered. The filtrate was extracted with water (2 × 25 mL) and the amino acid concentration in the aqueous extract determined by UV (257 nm for phenylalanine, 275 nm for tyrosine, 278 nm for tryptophan, and 250 nm for cysteine). Solubilities thus obtained were corrected for the solubilities of the amino acids in *n*-hexane (determined in the same way without surfactant).

*(6,6'-Hexadecylimino)bis(6-deoxy-1,2-*O*-isopropylidene)-α-*D*-glucofuranose* (**6**).—A well-mixed mixture of compound **1** [15] (202 mg, 10 mmol) and **2** (121 mg, 5 mmol) was heated at 110–120°C for 1 h without solvent. Purification of the dark residue by column chromatography on silica gel (EtOAc) afforded **6** (261 mg, 80.7%) as a syrup; $[\alpha]_D^{27}$ +11.6 (*c* 1.0, $CHCl_3$); ^{1}H NMR (200 MHz); δ 0.88 (t, 3 H, CH_3), 1.28 (m, 34 H, CH_2 and CH_3), 1.48 (s, 6 H, CH_3), 2.68–2.80 (m, 4 H, NCH_2 and H-6,), 2.93 (d, 2 H, H-6,), 3.92 (d, 2 H, H-4), 4.05 (d, 2 H, H-5), 4.31 (s, 2 H, H-3), 4.51 (d, 2 H, J = 3.5 Hz, H-2), 4.79 (br, 4 H, OH), 5.92 (d, 2 H, J = 3.5 Hz, H-1); ^{13}C NMR (200 MHz) δ 14.1 (CH_3), 22.7–31.8 (CH_2 and CH_3), 56.1 (NCH_2), 58.2 (C-6), 66.1 (C-5), 74.6

(C-3), 81.5 (C-4), 85.0 (C-2), 104.9 (C-1), 111.6 (Me_2C of -1, 2); (M^+) ion m/z 631 ($M^+ - 15$).

*6-Deoxy-6-*N*-tosyl-hexadecylamino-1,2-*O*-isopropylidene-α-*D*-glucofuranose* (**7**).—A well mixed mixture of **1** (404 mg, 2 mmol) and *N*-hexadecyl-*p*-toluenesulfonamide [20] (**3**) (791 mg, 2 mmol) was heated for 2 h at 120–130°C without solvent. Purification of the crude product by column chromatography on silica gel (1 : 1 AEtOAc–CH_2Cl_2) afforded **7** (836 mg, 70% based on **1** consumed); mp 63°C; $[\alpha]_D^{27} - 17.3°$ (*c* 1.2, $CHCl_3$); ν (KBr) cm^{-1} 3450 (OH), 3070 (ArC–H_{st}), 1385, 1370 (gem dimethyl), 1335 (SO_2 as), 1155 (SO_2 sy), 1090, 1070, 1010 (COH and ether COC); UV (EtOH) λ_{max} log ϵ 211.8 (4.11); ^{1}H NMR (200 MHz) δ 0.88 (t, 3 H, CH_3), 1.22–1.30 (m, 31 H, CH_2 and CH_3), 1.48 (s, 3 H, CH_3), 2.43 (s, 3 H, PhCH_3), 3.07–3.42 (m, 4 H, NCH_2 and H-6,6′), 3.60 (br, 1 H, OH), 3.90–4.14 (brm, 3 H, H-5, H-4 and OH), 4.41 (s, 1 H, H-3), 4.53 (d, 1 H, J = 3.5 Hz, H-2), 5.92 (d, 1 H, J = 3.5 Hz, H-1), 7.31 and 7.70 (AB q, 4 H, J = 8.0 Hz, Ar); ^{13}C NMR (200 MHz) δ 14.1 (CH_3), 21.4 (PhCH_3), 22.6–31.9 (CH_2 and CH_3), 50.5 (NCH_2), 52.7 (C-6), 68.8 (C-5), 75.0 (C-3) 81.1 (C-4), 85.0 (C-2), 105.2 (C-1), 111.6 (Me_2 of −1, 2), 127.3, 129.7, 135.9 and 143.5 (Ar). Anal. Calcd for $C_{32}H_{55}NO_7S$: C, 64.28; H, 9.28; N, 2.34. Found C, 64.52; H, 9.48; N, 2.42.

N-Benzylhexadecylamine (**4**).—Hexadecylamine (2.41 g, 10 mmol) and freshly distilled benzaldehyde (1.06 g, 10 mmol) was added to EtOH (30 mL) and the solution was boiled under reflux for 2 h at 100°C. The solution was cooled for 2 h to room temperature. Sodium borohydride (700 mg, 18 mmol) was added during 10 min and the mixture was stirred for 30 h at room temperature. The excess sodium borohydride was decomposed with water (4.0 mL) and the solvent evaporated under diminished pressure. The residue was dissolved in CH_2Cl_2 (200 mL) and washed with water (20 mL). The organic solution was dried (Na_2SO_4) and concentrated to give the crude product. Purification by column chromatography on silica gel (1 : 4 EtOAc–CH_2Cl_2) afforded **4** as a semi-solid product, which was crystallized from EtOH (2.27 g, 68.5%); mp 37–38°C; ^{1}H NMR (60 MHz) δ 0.9 (t, 3 H, CH_3), 1.28 (s, 28 H, CH_2), 1.7 (br, 1 H, NH), 2.67 (t, 2 H, CH_2N), 3.67 (s, 2 H, NCH_2Ph), 7.17 (s, 5 H, Ar). Anal. Calcd for $C_{23}H_{41}N$: C, 83.31; H, 12.46; N, 4.22. Found C, 83.20; H, 12.53; N, 4.48.

*6-Deoxy-6-(*N*-benzylhexadecyl)amino-*1,2-*O*-isopropylidene-α-*D*-glucofuranose* (**8**). —Compound **4** (1.65 g, 5 mmol) was heated to 100°C and to it was added **1** (1.01 g, 5 mmol) and the melt mixed with a sealed capillary tube. The temperature was raised to 130°C and kept for 1 h. Purification of the dark product by column chromatography on silica gel (1 : 1 EtOAc–CH_2Cl_2) afforded **8** (1.70 g, 64.2%); mp 64°C; $[\alpha]_D^{27} + 21.8°$ (*c* 1.0, $CHCl_3$); UV (hexane) λ_{max} log ϵ 213.0 (3.83); ^{1}H NMR (200 MHz) δ 0.88 (t, 3 H, CH_3), 1.24–1.34 (m, 31 H, CH_2 and CH_3), 1.46 (s, 3 H, CH_3), 2.50–2.69 (m, 3 H, NCH_2 and H-6′), 2.80 (dd, 1 H, J = 4.3 Hz, H-6), 3.68 (q, 2H, NCH_2Ph), 3.94 (dd, 1 H, J = 2.4, 2.5 Hz, H-4), 4.0 (m, 1 H, H-5), 4.25 (d, 1 H, J = 2.4 Hz, H-3), 4.49 (d, 3 H, J = 3.7 Hz, H-2 and OH), 5.91 (d, 1 H, J = 3.6 Hz, H-1), 7.29 (m, 5 H, Ar), ^{13}C NMR (200 MHz) δ 14.1 (CH_3), 22.6–31.8 (CH_2 and CH_3), 54.0 (NCH_2Ph), 56.7 (NCH_2), 58.5 (C-6), 66.0 (C-5), 75.0 (C-3), 81.6 (C-4), 85.0 (C-2), 104.8 (C-1), 111.3 (Me_2C of −1, 2), 127.4, 128.4, 129.2 and 137.4 (Ar); (M^+) ion m/z 531,518 ($M^+ - 15$). Anal. Calcd for $C_{32}H_{55}NO_5$: C, 72.0; H, 10.39; N, 2.62. Found C, 72.34; H, 10.11; N, 2.72.

N,N′-*Di(hexadecyl)*-p-*phenylenediamine* (**5**).—Terephthaldehyde (670 mg, 10 mmol) and hexadecylamine (2.41 g, 10 mmol) were dissolved in EtOH (30 mL) and refluxed for 2 h. After cooling to room temperature $NaBH_4$ (1.10 g, 30 mmol) was added during 15 min. The solution was stirred for 30 h, excess reductant decomposed with water (5 mL) and the solvent evaporated under diminished pressure. The residue was dissolved in CH_2Cl_2 (200 mL), and washed with water (20 mL). The organic solution was dried (Na_2SO_4) and concentrated to give a semi-solid residue. Purification by column chromatography on silica gel (1:2 EtOAc–CH_2Cl_2) afforded **5** as a semi-solid product (3.68 g, 63%); ^{1}H NMR (200 MHz) δ 0.88 (t, 6 H, CH_3), 1.26 (s, 54 H, CH_2), 1.48 (br, 2 H, NH), 2.61 (t, 4 H, NCH_2), 3.73 (s, 4 H, CH_2Ph), 7.23 (s, 4 H, Ar).

*[6,6′-(*N,N′-*Di(hexadecyl)*-p-*phenylenediamino)]bis(6-deoxy-1,2-*O-*isopropylidene)-α-*D-*glucofuranose* (**9**).—Compound **5** (1.17 g, 2 mmol) was heated to 100°C and to it was added **1** (808 mg, 4 mmol). The temperature was raised to 120–130°C and kept for 2 h. Purification of the residue by column chromatography on silica gel (1:2 EtOAc–CH_2Cl_2) afforded flakes, which were crystallized from MeCN to give **9** (1.25 g, 63%); mp 57–58°C; $[\alpha]_D^{27}$ + 23.7°C (*c* 1.0, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 223.4 (3.75); ^{1}H NMR (200 MHz) δ 0.88 (t, 3 H, C*H*$_3$), 1.26–1.29 (m, 62 H, CH_2 and CH_3), 1.47 (s, 6 H, CH_3-a), 1.54 (br, 2 H, OH), 2.58 (brm, 8 H, CH_2N, H-6′ and OH), 2.9 (d, 2 H, H-6), 3.63 (m, 6 H, NC*H*$_2$Ph and H-4) 3.91 (s, 2 H, H-5), 4.17 (s, 2 H, H-3), 4.46 (d, 2 H, J = 3.4 Hz, H-2), 5.91 (d, 2 H, J = 3.4 Hz, H-1), 7.26 (s, 4 H, Ar); ^{13}C NMR (200 MHz) δ 15.3 (CH_3), 22.7–31.9 (CH_2 and CH_3), 55.7 (NCH_2Ph), 57.4 (NCH_2CH_2), 58.9 (C-6), 66.6 (C-5), 75.3 (C-3), 81.4 (C-4), 85.2 (C-2), 104.9 (C-1), 111.4 and 112.0 (2X Me_2C of -1, 2), 129.4 and 137.8 (Ar). Anal. Calcd for $C_{58}H_{104}N_2O_{10}$: C, 70.40; H, 10.59; N, 2.83. Found C, 70.75; H, 10.71; N, 2.92.

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References

[1] M.N. Jones, *Chem. Soc. Revs.*, (1992) 127–135.
[2] V.A. Lutsenko, O.P. Korenkova, and R.M. Panich, *Neftepererab. Neftekhim*, (1968) 31–34 (in Russian).
[3] V.A. Lutsenko, R.M. Panich, O.P. Korenkova, and M.Ya. Musenko, *Kolloid Zh.*, 31 (1969) 86–91 (in Russian).
[4] M.R. Jenner and R. Khan, *J. Chem. Soc., Chem. Commun.*, (1980) 50–51.
[5] S. Bottle and I.D. Jenkins, *J. Chem. Soc., Chem. Commun.*, (1984) 385.
[6] W. Engst, A. Elsher, H. Schliemann, and G. Mieth, *Nahrung*, 34 (1990) 857–867.
[7] K. Hess and E. Messmer, *Chem. Ber.*, 54B (1921) 499–506.
[8] R.C. Chalk, D.H. Ball, and L. Long, Jr., *J. Org. Chem.*, 31 (1966) 1509–1514.
[9] D. Loganathan, A.J. Amonkar, and G.K. Trivedi, *Ind. J. Chem.*, 22B (1983) 400–401.
[10] M. Therisod and A.M. Klibanov, *J. Am. Chem. Soc.*, 108 (1986) 5638–5640.
[11] D. Plausqueelec, F. Roulleau, F. Bertho, and M. Lefeuvre, *Tetrahedron*, 42 (1986) 2457–2460.
[12] S. Riva, J. Chopineau, A.P.G. Kieboom, and A.M. Klibanov, *J. Am. Chem. Soc.*, 110 (1988) 584–589.

[13] K. Adelhorst, F. Bjorkling, S.E. Godfredsen, and O. Kirk, *Synthesis*, (1990) 112–115.
[14] P.Y. Goueth, P. Godalis, R. Bikanga, P. Gode, D. Postel, G. Ronco, and P. Villa, *J. Carbohydr. Chem.*, 13 (1994) 249–272.
[15] H. Ohle and L. Vargha, *Berichte*, 62 (1921) 2435–2444.
[16] R.E. Parker and N.S. Issacs, *Chem. Revs.*, 59 (1959) 737–799.
[17] R.J. Abraham, L.D. Hall, L. Hough, and K.A. McLauchlan, *J. Chem. Soc.*, (1962) 3699–3705.
[18] D.M. Vyas, H.C. Jarell, and W.A. Szarek, *Can. J. Chem.*, 53 (1975) 2748–2754.
[19] W.B. Gratzer and G.H. Beaven, *J. Phys. Chem.*, 73 (1969) 2270–2273.
[20] R. Sasin, W.A. Buttle, A.L. Borror, and G.S. Sasin, *J. Oil Chem. Soc.*, 34 (1957) 358–360.