Effect of Various Factors on the Adsorption

Using Aerosil 200, the lower the temperature was, the more increased the amount adsorbed of PZS. (1.04×10^{-4} mole/g at 30°, 0.63×10^{-4} mole/g at 40°). Although the thermodynamic parameters of adsorption were not obtained, the result suggested that the adsorption of PZS by Aerosil might belong to physical adsorption type.

The fact that the amount adsorbed increased with pH may indicate that undissociated drug molecules are more liable to adsorb on Aerosil (1.04×10^{-4} mole/g at pH 7.0, 2.9×10^{-5} mole/g at pH 6.0, 1.5×10^{-5} mole/g at pH 5.2), as was observed in the case of carbon black.[5]

The more the concentration of sodium chloride and that of buffer solution increased, the more increased the amount adsorbed of PZS. This may indicate that water molecules around the surface of adsorbent are removed and drug molecules are adsorbed on those sites, as was observed in the case of carbon black and silica gel.[8]

The more the concentration of urea increased, the more decreased the amount adsorbed (1.04×10^{-4} mole/g at urea free, 2.6×10^{-5} mole/g at 2M, 1.5×10^{-5} mole/g at 4M). This decrease in adsorption may be explained on the consideration that the adsorption of PZS proceeded on the hydrophobic interaction.[5]

UV Absorption Spectroscopy by Reflection Method

UV absorption spectrum by reflection method of the adsorbent after the adsorption experiment gave a maximum at the same wave length as the case of drug only. The adsorbent without the adsorption experiment gave no maximal absorption. This result indicated that the drugs were adsorbed by Aerosil and it was considered possible that the adsorption of drugs by Aerosil may belong to the physical type.

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8) N. Nambu, T. Nagai, and H. Nogami, *Chem. Pharm. Bull.* (Tokyo), **19**, 1058 (1971).

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Anomeric Proton Signals of the Component Monosaccharides of Free Oligoglycosides in Nuclear Magnetic Resonance Spectroscopy

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Nuclear magnetic resonance (NMR) spectroscopy has become an important means for structure elucidation in carbohydrate chemistry. A number of works have been reported on the determination of the glycosidic configurations and conformations of the component sugar units in oligoglycosides by use of the chemical shifts and the vicinal coupling constants

1) Location: *Katakasu, 812, Fukuoka.*

of their anomeric protons. However, the determination is usually made indirectly[2] *via* the spectra of oligoglycoside-permethylates[3] and -peracetates[4] which are soluble in $CDCl_3$. It is due to the facts that the free compounds are poorly soluble in $CDCl_3$, CD_3OD, CD_3COCD_3, and D_2O, and that they show, on the spectra taken in the solutions of rather particular and expensive solvents such as C_5D_5N, DMF-d_7, and DMSO-d_6,[2] the anomeric protons as broad signals interupted by those of the remaining hydrogens on the carbon and oxygen atoms. The methods currently available as mentioned above have, however, another problem. Namely, the peracetates give the spectra where the hydrogens adjacent to the acetoxyl groups show their signals in the same region as those of the anomeric protons, while the permethylates, of which the anomeric protons resonate at unique positions widely separated from other resonance peaks, are not easily accessible in some cases, for instance cardiac glycosides.

This communication concerns a simple and convenient method to assign the anomeric proton signals of the component monosaccharides of free oligoglycosides in NMR spectroscopy.

Taking advantage of good solubility of oligoglycosides, even those of relatively large molecular size, in $CHCl_3$–MeOH or $CHCl_3$–MeOH–H_2O mixture, the spectra of dioscin,[5]

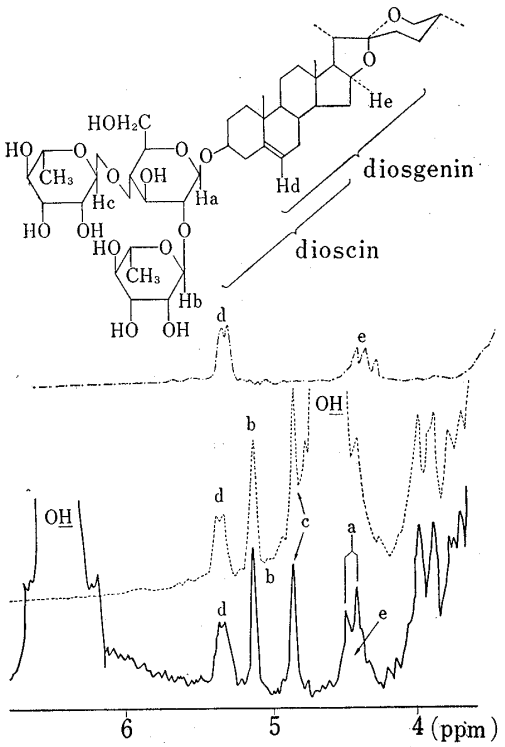

Fig. 1. NMR Spectra of Dioscin and Diosgenin

----------: dioscin in $CDCl_3$: CD_3OD: D_2O (7: 3: 0.5)
———: dioscin (15 mg) in $CDCl_3$: CD_3OD: D_2O (7 : 3: 0.5)+CF_3COOH (10%)
-·-·-·-: diosgenin in $CDCl_3$

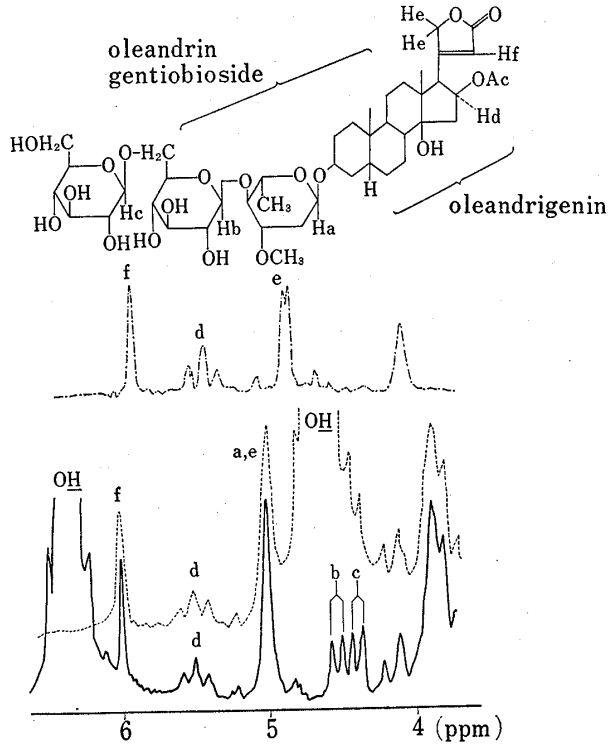

Fig. 2. NMR Spectra of Oleandrin Gentiobioside and Oleandrigenin

----------: oleandrin gentiobioside in $CDCl_3$: CD_3OD (1: 1)
———: oleandrin gentiobioside (15 mg) in $CDCl_3$: CD_3OD (1: 1)+CF_3COOH (10%)
-·-·-·-: oleandrigenin in $CDCl_3$

2) Kubinyi has reported a direct determination of the conformation of rhamnose in proscillaridin (3β,14β-dihydroxy-bufa-4,20,22-trienolide 3-O-α-L-rhamnopyranoside) by NMR spectra of free glycoside taken in $CDCl_3$/CD_3OD, tetrahydrofuran-d_8, acetone-d_6, DMSO-d_6, and DMSO-d_6/D_2O (H. Kubinyi, *Arch. Pharm.*, **304**, 701 (1971)).

3) *e.g.* T. Nohara, H. Yabuta, M. Suenobu, R. Hida, K. Miyahara, and T. Kawasaki, *Chem. Pharm. Bull.* (Tokyo), **21**, 1240 (1973); H. Okabe and T. Kawasaki, *ibid.*, **20**, 514 (1972).

4) *e.g.* A.K. Dzizenko, V.V. Isakov, N.I. Uvarova, G.I. Oshitok, and G.B. Elyakov, *Carbohydrate Research*, **27**, 249 (1973).

5) T. Kawasaki and T. Yamauchi, *Chem. Pharm. Bull.* (Tokyo), **10**, 703 (1962).

oleandrin gentiobioside,[6] Akebia seedsaponin E,[7] and pharbitic acid C[8] were measured in $CDCl_3$–CD_3OD[2] or $CDCl_3$–CD_3OD–D_2O mixture. The anomeric protons of sugar moiety gave satisfactorily sharp signals lieing in 4.2—5.5 ppm, but the hydroxyl hydrogens[9] also appeared in the same region.

Hall[10] recommended in a NMR study of oligosaccharides to deuterate all labile hydrogen atoms with D_2O and subsequently to shift the HOD peak by altering the pH. He also demonstrated,[10] on a spectrum of 5,6-anhydro-1,2-O-isopropylidene-α-D-glucofuranose in acetone, a successful removal of the interference of C_3–OH on the C_2–H and C_3–H resonances with a trace of trifluoroacetic acid.

Accordingly trifluoroacetic acid[11] was added to the aforementioned solution up to 5—

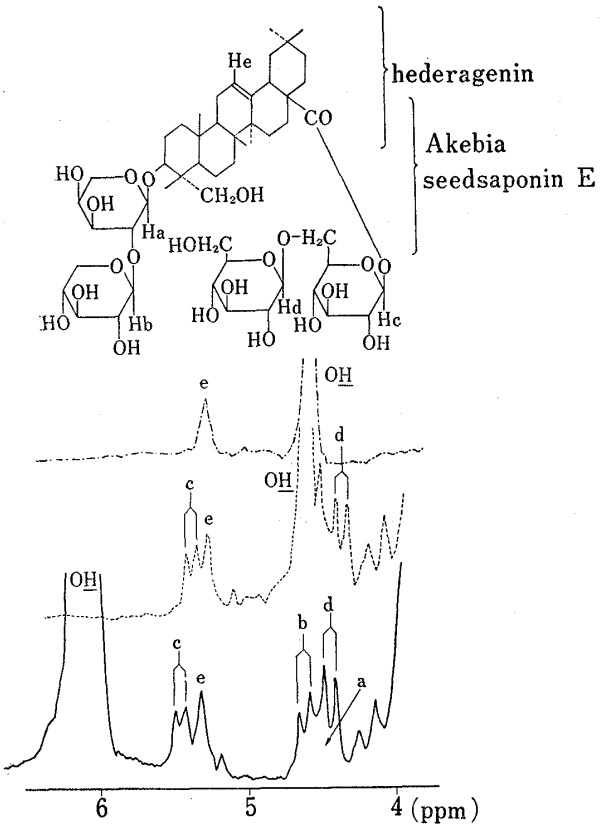

Fig. 3. NMR Spectra of Akebia Seedsaponin E and Hederagenin

----------: Akebia seedsaponin E in $CDCl_3$:CD_3OD:D_2O (2:3:1)
————: Akebia seedsaponin E (15 mg) in $CDCl_3$: CD_3OD: D_2O (2:3:1)+CF_3COOH (10%)
-·-·-·-: hederagenin in $CDCl_3$: CD_3OD (1:1)

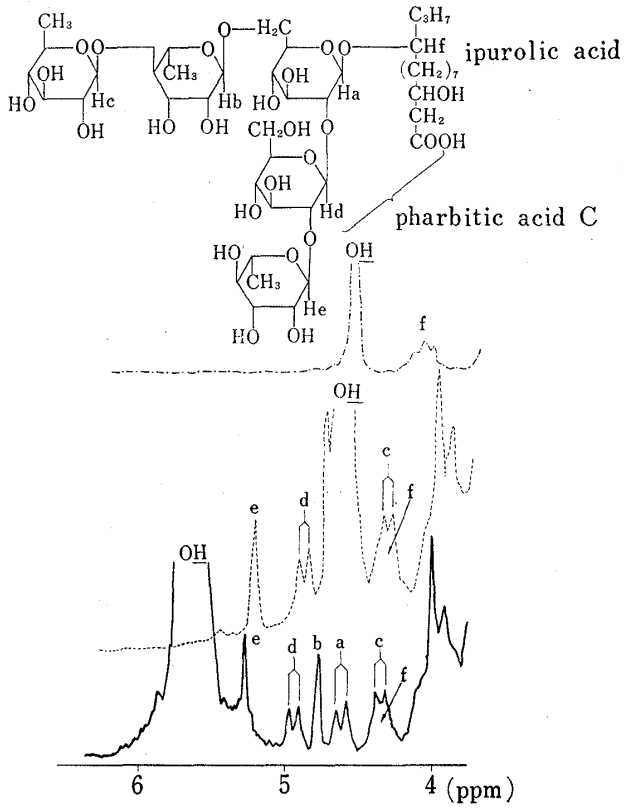

Fig. 4. NMR Spectra of Pharbitic Acid C and Ipurolic Acid

----------: pharbitic acid C in $CDCl_3$: CD_3OD: D_2O (2:3:1)
————: pharbitic acid C (15 mg) in $CDCl_3$: CD_3OD: D_2O (2:3:1)+CF_3COOH (15%)
-·-·-·-: ipurolic acid in $CDCl_3$

6) T. Yamauchi, N. Takada, and T. Mimura, Abstracts of Papers, 93rd Annual Meeting of Pharmaceutical Society of Japan, Tokyo, April, 1973, II, p. 222.
7) R. Higuchi and T. Kawasaki, *Chem. Pharm. Bull.* (Tokyo), **20**, 2143 (1972).
8) H. Okabe and T. Kawasaki, *Chem. Pharm. Bull.* (Tokyo), **20**, 514 (1972).
9) Including the hydrogen of DOH which is formed when $CDCl_3$–CD_3OD–D_2O mixture is used as solvent.
10) L.D. Hall, "Advances in Carbohydrate Chemistry," Vol. 19, ed. by M.L. Wolfrom and R.S. Tipson, Academic Press, New York and London, 1964, p. 82, 64, 52.
11) It is an excellent solvent for various organic compounds (Y. Kawazoe and M. Ohnishi, *Chem. Pharm. Bull.* (Tokyo), **11**, 243 (1963)).
12) The amount of CF_3COOH is desirable to be varied depending on the number of hydroxyl groups in the oligoglycoside, so that the signals of hydroxyl hydrogens shift downward by 1.5—2 ppm.
13) In these conditions oligoglycosides were not affected by CF_3COOH. Hydroxyl signals shifted back to the anomeric proton region after several hours probably owing to the formation of CF_3COOCD_3.

15%[12] and the spectra were taken at room temperature (23°) within a few hours.[13] As shown in Fig. 1—4, in each case the resonances of the interfering hydrogens were displaced to much lower field than those of anomeric protons which remained almost in the original field. Olefinic proton(s) and/or hydrogen(s) next to ether oxygen, if any, in the aglycone gave the signal(s) also in the region 4.2—5.5 ppm, but they could be distinguished by comparison with the spectrum of the aglycone. Therefore all the anomeric proton signals of the component monosaccharides were easily and clearly located on the spectrum.

The method has so far been applied to thirty-five known oligoglycosides[14] (steroid saponins 8, cardiac glycosides 7, pregnane glycosides 4, hydroxyfatty acid glycosides 4) and it seems to be generally applicable.

Experimental

A sample (10—15 mg) was dissolved in 0.4 ml of a mixture of $CDCl_3$–CD_3OD or $CDCl_3$–CD_3OD–D_2O, and CF_3COOH was added up to 5—15%.[13] NMR spectra were taken at room temperature (23°) within a few hours on a JEOL PS-100 (100 MHz) spectrometer using tetramethylsilane as internal reference.

14) Mostly isolated and characterized in this laboratory. Five cardiac glycosides including oleandrin gentiobioside (ref. 6) and three pregnane glycosides were kindly supplied by Prof. T. Yamauchi of Fukuoka University, to whom the authors' thanks are due.

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Hypotensive and Radioprotective Properties of N^6-Substituted Adenosine Derivatives

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Previous studies of Asakura[2,3] and of ours[4,5] have been concerned with N^6-substituted adenosines which are effective in protecting experimental animals from deleterious actions of ionizing radiations. It seems likely that the radioprotective action of these compounds is attributable partly to hypoxia caused secondarily by their cardiovascular actions,[3] and partly to their direct radioprotective action on cellular components.[4,5] The present paper reports similar radioprotective action of several other adenosine derivatives. N^6-(*p*-Hydroxyphenylethyl)adenosine (**1**) and N^6-(indole-3-ethyl)adenosine (**2**) are the compounds of choice as they possess the structure of tyramine and tryptamine in the molecule. Special attention has been directed to the hypotensive action of these compounds, because neither of the compounds has been reported thus far in this respect. Adenosine has been known as a potent vasodilator.[6]

1) Location: *Anagawa 4-9-1, Chiba-shi.*
2) H. Asakura, *Hoshasen Seibutsu Kenkyu*, **4**, 128 (1969).
3) H. Asakura, K. Lee, S. Ikegami, M. Shikita, and S. Akaboshi, *J. Radiat. Res.*, **15**, 19 (1974).
4) Y. Takagi, F. Sato, M. Shikita, and S. Akaboshi, *Chem. Pharm. Bull.* (Tokyo), **18**, 2514 (1970).
5) Y. Takagi, M. Okazaki, and M. Shikita, *J. Radiat. Res.*, **12**, 100 (1971).
6) R.B. Philp and V. Lemieux, *Nature*, **218**, 1072 (1968).