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# LASER-RAMAN SPECTROSCOPIC STUDY OF CARBOHYDRATES: 1-THIO-β-D-HEXOPYRANOSIDES

ANTHONY T. TU*, JAMINE LEE†, AND YUAN CHUAN LEE**
*Departments of Biochemistry* and Physics†, Colorado State University, Fort Collins, Colorado 80523, and **Department of Biology and McCollum-Pratt Institute, Johns Hopkins University, Baltimore, Maryland 21218 (U. S. A.)*

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## ABSTRACT

Some β-D-hexopyranosides of 1-thio-D-glucose, 2-acetamido-2-deoxy-1-thio-D-glucose, and 1-thio-D-galactose were examined by laser-Raman spectroscopy. An anomeric C–H bending vibration was found at 891 ±7 $cm^{-1}$ for all compounds investigated; thus, the anomers of these sugars can be differentiated by Raman spectroscopy. The *N*-acetyl group and carboxyl group can also be detected by Raman spectroscopy. Unlike protein samples, the carbohydrates in aqueous solution yield less useful information from Raman spectra than in the solid state; this is due to the extensive overlapping of carbohydrate OH bands with water OH bands.

## INTRODUCTION

Extensive studies have been made in attempts to correlate structural information on carbohydrates with their infrared (i.r.) spectra[1], and some success has been attained in such areas as the determination of the configuration of the glycosidic linkage. Although laser-Raman spectroscopy has recently become a powerful tool in the structural study of proteins, nucleic acids, and other biological compounds, few such studies on carbohydrates have been reported. Raman spectroscopy has several inherent advantages over infrared spectroscopy; for example, Raman spectra can be obtained from a solid sample directly, without mixing it with Nujol or preparing KBr pellets (as in the case of i.r. spectroscopy). Moreover, Raman spectra can usually be obtained for aqueous solutions, because of relatively weak interference from the OH vibration of the water molecule, and absorption peaks in Raman spectra are often sharper than those in the corresponding i.r. spectra[2,3] and thus provide more-precise information.

However, i.r. and Raman spectroscopy can be complementary to each other. In order to obtain a complete roster of the vibrational modes of carbohydrates, it is advantageous to have information from both infrared and Raman spectra, because certain bondings, such as C=N, C=S, C–C, and SH, are manifested strongly in Raman spectra, whereas the corresponding bands are weak in i.r. spectra.

Laser-Raman spectra of glycosidic linkages have been extensively studied by Tu *et al.*[2,3]. Typically, α-D anomers show a band at 840 $cm^{-1}$, and β-D anomers, at 890 $cm^{-1}$; these originate[4,5] from deformation of anomeric C–H. [Anomeric C–H is equatorial for an α-D anomer, and axial for a β-D anomer, in the $^4C_1$(D) conformation.]

It was of interest to examine whether this is true for 1-thioglycosides, in which the glycosidic oxygen-atom is replaced by a sulfur atom. Some 1-thioglycosides have been used as models in important biological systems; for example, 1-thiogalactosides have been used for the induction of glycosidases[6], for affinity chromatography[7,8], for cell-adhesion studies[9], and for preparation of neoglycoproteins[10]. An instance of a 1-thioglycoside involved in a carbohydrate–peptide linkage (*S*-D-glucosyl-L-cysteine) has been reported.

In view of these examples, it was desirable to acquire precise, structural information on such compounds. As the initial step towards such an objective, the following compounds were examined by laser-Raman spectroscopy: methyl 1-thio-β-D-galactopyranoside (**1**) [galactosyl-S-$CH_3$ (β)], carboxymethyl 1-thio-β-D-galactopyranoside (**2**) [galactosyl-S-$CH_2CO_2H$ (β)], carboxymethyl 1-thio-β-D-glucopyranoside (**3**) [glucosyl-S-$CH_2CO_2H$ (β)], cyanomethyl 2-acetamido-2-deoxy-1-thio-β-D-glucopyranoside (**4**) [GlcNAc-S-$CH_2CN$ (β)], and 2-(acrylamidomethylaminocarbonyl)ethyl 1-thio-β-D-glucopyranoside (**5**) [glucosyl-S-$(CH_2)_2CONHCH_2NHCOCH{=}CH_2$ (β)].

## EXPERIMENTAL

*Synthesis of 1-thiohexopyranosides.* — 1-Thiohexopyranosides **2**, **3**, and **4** were prepared by the method described previously[11]. Methyl 1-thio-β-D-galactopyranoside (**1**) was synthesized from 2-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-2-thiopseudourea hydrobromide and methyl iodide by an analogous method. Compound **5** was synthesized by treating *N,N'*-methylenebis(acrylamide) with 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranose[12].

*Laser-Raman spectroscopy.* — Laser-Raman spectra were obtained by excitation with the 514.5-nm line of an argon-ion laser (Spectrophysics, model Sp-164) having a green interference filter, and recorded on a Spex Ramalog 5. Samples were examined in the solid phase (in powder form), and in aqueous solution. Capillary cells (1.0-mm inside diam.) were filled with ~10 μl of aqueous solution (concentration 13%). Spectra were recorded in the range of 3800 to 200 $cm^{-1}$.

## RESULTS AND DISCUSSION

*Glycosidic linkages.* — Determination of anomeric configuration is an important aspect of structural carbohydrate chemistry, and is frequently achieved by $^1$H-n.m.r. spectroscopy, measurement of optical rotation, or i.r. absorption spectroscopy. As i.r. spectroscopy is closely related to Raman spectroscopy, a brief review of salient features of the i.r. spectra of anomers will be given.

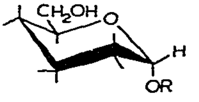
It is generally accepted that several absorption bands in i.r. spectra are characteristic for α or β anomers, and they have been grouped as types 1, 2a, 2b, and 3 (see Table I). The origin of type 1 bands was assigned to "ring asymmetrical vibration", similar to that of 1,4-dioxane[4,5,11]. Type 2 bands originate from the anomeric C–H deformation (bending vibration): equatorial C–H vibration in α-D anomers in the $^{4}C_{1}$(D) conformation. Type 3 vibrational bands are the result of "symmetrical ring vibration" of the hexopyranose ring. These three types of absorption band of α and β anomers overlap considerably, and only type 2 bands can be used to differentiate the anomeric glycosidic linkages. Recent studies[13] of carbohydrates by laser-Raman spectroscopy also led to the conclusion that the type 2 band can be useful for discerning anomeric linkages.

TABLE I

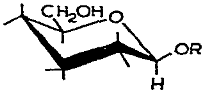
LASER-RAMAN FREQUENCIES CORRESPONDING TO INFRARED-SPECTRAL CHARACTERISTICS OF ANOMERS

| *Compounds* | *Type 1* | *Type 2 (a or b)* | *Type 3* |
|---|---|---|---|
| **Infrared** | | | |
| α anomers | 917 ±13 | 844 ±8 | 766 ±10 |
| β anomers | 920 ±5 | 891 ±7 | 744 ±9 |
| **Raman** | | | |
| 3 (β) | | | |
| solid | 921 | 895 | 795 |
| $H_2O$ solution | n.o.[a] | 898 | n.o. |
| 1 (β) | | | |
| solid | 939 | 884 | 775 (weak) |
| $H_2O$ solution | 920 (sh[b]) | 883 (sh) | n.o. |
| 2 (β) | | | |
| solid | 916 | 895 | 795 (weak) |
| $H_2O$ solution | n.o. | 902 | n.o. |
| 5 (β) | | | |
| solid | n.o. | 890 | n.o. |
| 4 (β) | | | |
| solid | n.o. | 884 | n.o. |
| $H_2O$ solution | n.o. | 887 (broad) | n.o. |

[a] n.o. = band not observed. [b] sh = shoulder.

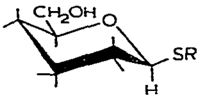


α anomer

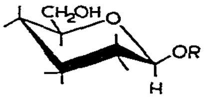


β anomer

We have now extended such studies to 1-thioglycosides. The results of the present laser-Raman spectroscopic investigation of five 1-thioglycosides in the solid state and in aqueous solution are summarized in Table I. Unlike i.r. spectra, Raman spectra either do not consistently show types 1 and 3 bands, or do not show them clearly. As in i.r. spectroscopy, only type 2 bands can be used for the analysis of α and β anomeric linkages. These Raman bands are very sharp, and are much superior in quality to those in the corresponding infrared spectra. As with glycosides, a distinct type-2b band appears at ~891 ±7 cm$^{-1}$ for all of the 1-thio-β-D-glycosides investigated (see Figs. 1, 2, and 3): this is reasonable, because the type 2b bands arise from the axial, C–H bond deformation, and appear not to be dependent on whether oxygen or sulfur is attached to C-1 of the glycosyl group. The type 2 bands of all of



β anomer



β anomer

TABLE II

CHARACTERISTIC, ANOMERIC, C–H BANDS IN THE LASER-RAMAN SPECTRA OF A VARIETY OF CARBOHYDRATES

| *Carbohydrate* | *Type 2a band* | *Type 2b band* | *Reference* |
|---|---|---|---|
| 3 (β) | | | |
| solid | — | 895 | this work |
| aqueous solution | — | | |
| 1 (β) | | | |
| solid | — | 884 | this work |
| aqueous solution | | | |
| 2 (β) | | | |
| solid | — | 895 | this work |
| aqueous solution | — | 902 | |
| 5 (β) | | | |
| solid | | 890 | this work |
| aqueous solution | | | |
| 4 (β) | | | |
| solid | — | 883 | this work |
| aqueous solution | — | 887 | |
| Methyl α-D-glucoside (solid) | 842 | — | 3 |
| Methyl β-D-glucoside (solid) | — | 890 | 3 |
| Deuterated[a] methyl α-D-glucoside (solid) | 837 | — | 3 |
| Deuterated[a] methyl β-D-glucoside (solid) | — | 850 | 3 |
| α-D-Glucose (solid) | 842 | — | 2 |
| | 840 | — | 17 |

TABLE II (*continued*)

| *Carbohydrate* | *Type 2a band* | *Type 2b band* | *Reference* |
|---|---|---|---|
| Deuterated[a] α-D-glucose (solid) | 842 | — | 2 |
| A mixture of 2-amino-2-deoxy-α- and -β-D-glucose (solid) | 865 | 896 | 2 |
| A mixture of deuterated[a] 2-amino-2-deoxy-α- and β-D-glucose (solid) | 845 | 905 | 2 |
| 2-Acetamido-2-deoxy-α-D-glucose (solid) | 865 | — | 2 |
| α-Lactose (solid) | 850 | — | 18 |
|  | 840 | — | 19 |
| β-Lactose (solid) | — | 890 | 18 |
|  | — | 898 | 19 |
| A mixture of α- and β-lactose (aqueous solution) | 855 | 890 | 18 |
| Deuterated[a] 2-acetamido-2-deoxy-α-D-glucose | 857 | — | 2 |
| Hyaluronic acid | — | 896 | 3 |

[a] All hydroxyl groups were isotopically exchanged to give -O–$^2$H.

the carbohydrates examined by laser-Raman spectroscopy are summarized in Table II.

*Comparison of solid samples and aqueous solutions.* — Because of the relatively weak background from water, the Raman spectra of many compounds can be measured in aqueous solution; this is usually considered to be the greatest advantage of Raman over infrared spectroscopy. However, in this regard, caution must be exercised in the interpretation of Raman spectra. Although the water background may be weak, its OH vibration is still evident in Raman bands (see Fig. 3). The OH stretching-vibration of a water molecule has a broad band from 3800 to 2800 cm$^{-1}$. For carbohydrates, the CH and OH stretching-vibration bands are also located in this region: thus, the water background completely obscures the carbohydrate bands, and this renders the Raman spectra of carbohydrates in this region almost useless. On the other hand, Raman spectra of carbohydrates can be useful in the region of 1800 to 300 cm$^{-1}$. However, even here, extreme caution is necessary, as spectra are considerably influenced by the water background. For instance, for aqueous solutions of **1**, there is a band at 1635 cm$^{-1}$ (see Fig. 2) that is not observed for solid samples thereof; this is due to the water background, and not to the carbohydrate (see Fig. 3).

In an aqueous solution of a glycoside, the glycosidic C–H deformation band at 890 cm$^{-1}$, and many other bands, become less distinct and unreliable. The broadness of carbohydrate bands in aqueous solution is partly due to water itself, but also partly due to extensive interaction of the hydroxyl groups of the carbohydrate with water. With proteins, this is not usually a problem, and excellent Raman spectra of proteins can be obtained for aqueous solutions thereof.

Therefore, for carbohydrates, the best results from Raman spectra are obtained with solid samples. Here again, Raman spectroscopy holds an advantage over i.r.

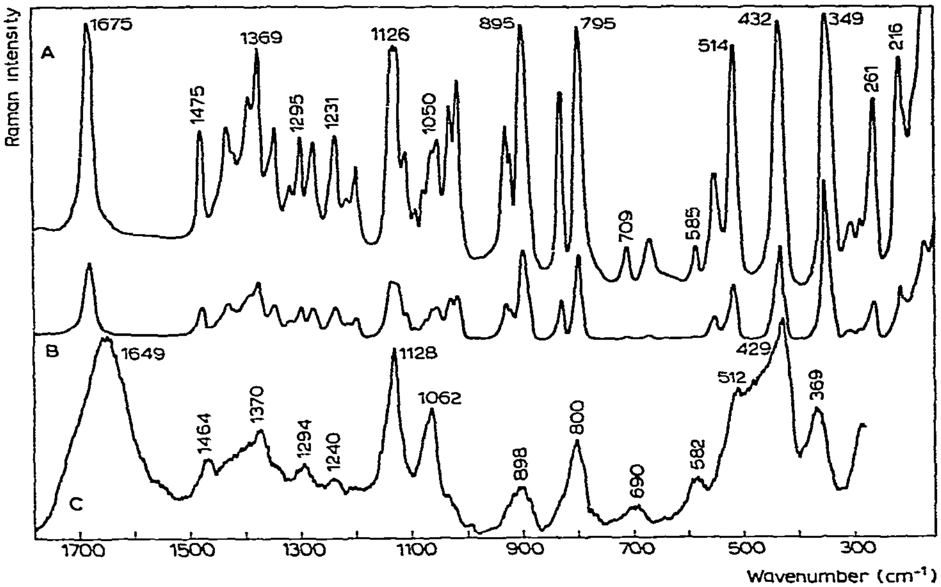


Fig. 1. Laser-Raman spectra of 3 in the range of 1800 to 200 $cm^{-1}$. (A, solid phase, radiant power 300 mW, resolution ±5 $cm^{-1}$; B, solid phase, radiant power 250 mW, resolution ±5 $cm^{-1}$; C, in aqueous solution, radiant power 500 mW, resolution ±6 $cm^{-1}$; all at integration time 0.2 sec, scanning speed 0.2 $cm^{-1}.sec^{-1}$.)

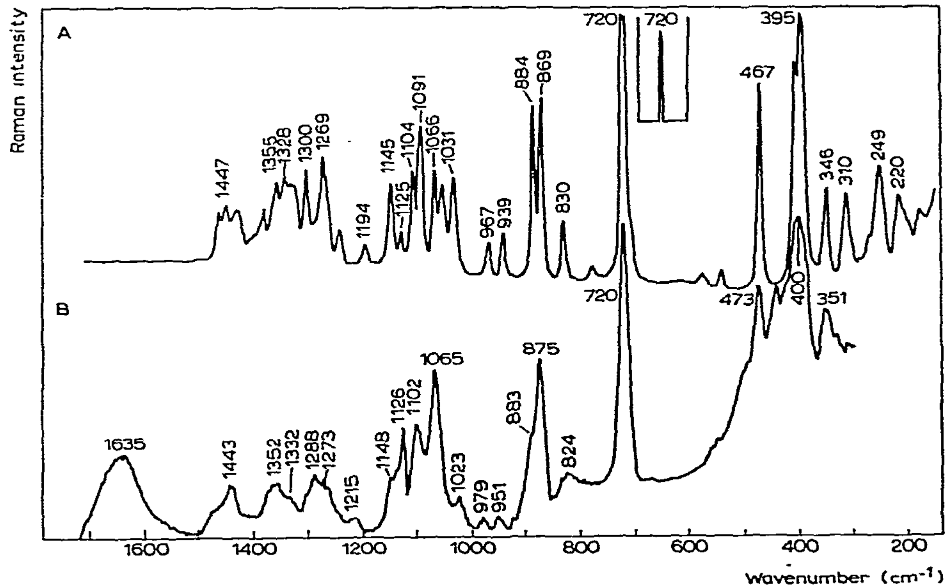


Fig. 2. Laser-Raman spectra of 1 in the range of 1700 to 200 $cm^{-1}$. (A, solid phase, radiant power 300 mW, resolution ±5 $cm^{-1}$; B, in aqueous solution, radiant power 500 mW, resolution ±6 $cm^{-1}$; both at integration time 0.2 sec, scanning speed 0.2 $cm^{-1}.sec^{-1}$.)

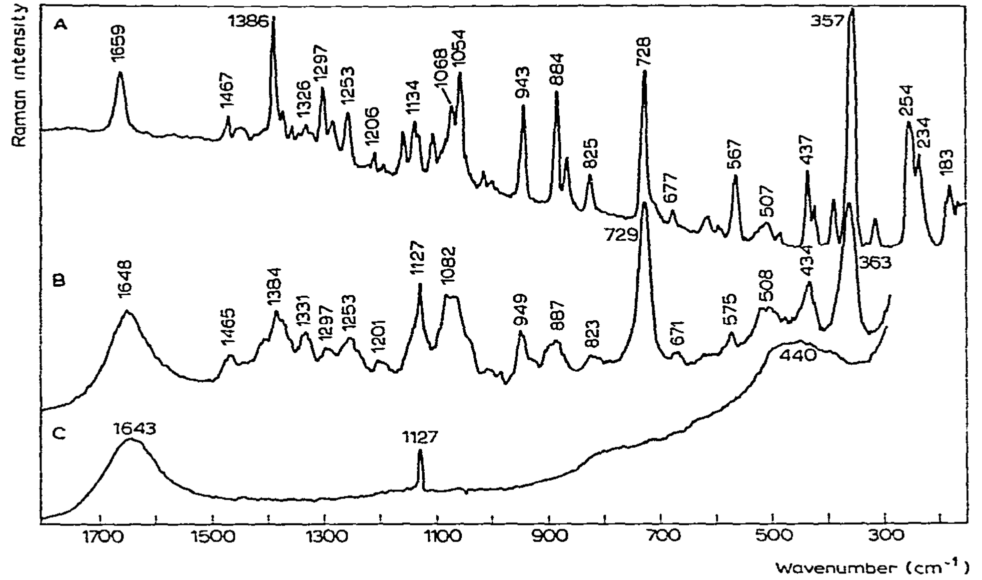
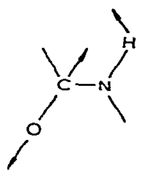


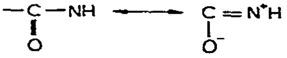
Fig. 3. Laser-Raman spectra of 4 and of water in the range of 1800 to 150 cm$^{-1}$. (A, solid phase radiant power 300 mW, resolution ±5 cm$^{-1}$; B, in aqueous solution, radiant power 500 mW, resolution ±6 cm$^{-1}$; C, water blank, radiant power 500 mW, resolution ±6 cm$^{-1}$; all at integration time 0.2 sec, scanning speed 0.2 cm$^{-1}$.sec$^{-1}$. The line at 1127 cm$^{-1}$ is due to the grating ghost of the instrument.)

spectroscopy, because of its simplicity of operation, with no need for preparation of samples in Nujol or KBr. Moreover, Raman spectra of solid carbohydrates give much sharper and more-distinct bands than those in infrared spectra.

N-*Acetyl group.* — The presence of 2-acetamido-2-deoxy-D-glucose and -galactose is quite common in Nature, and the *N*-acetyl group can be readily detected in a solid sample at 1659 cm$^{-1}$ (see Fig. 3). There are no bands from 1800 to 1500 cm$^{-1}$ in the spectra of most of the neutral carbohydrates, and identification of the *N*-acetyl group in solid samples of acetamidodeoxy carbohydrates can be achieved without ambiguity (with an aqueous solution, however, identification of the *N*-acetyl group is impossible). There is a band at 1648 cm$^{-1}$ for **4** (see Fig. 3) that is due to water background and that is present in the Raman spectra of aqueous solutions of all carbohydrates examined (see Figs. 1–3), but is absent from those of corresponding solid samples. The origin of the band for the *N*-acetyl group at 1659 cm$^{-1}$ is due to the in-plane vibration of –NHCO–.



The *N*-acetyl group, like the peptide bond in proteins, can exist in two resonance forms; it appears at the position of a normal, Amide I band.



$$\underset{\underset{O}{\|}}{-C}-NH \longrightarrow \underset{\underset{O^-}{|}}{C}=N^+H$$

In the Raman spectrum of 2-acetamido-2-deoxy-α-D-glucose[2], the *N*-acetyl band also appears at 1632 $cm^{-1}$, which is close to 1659 $cm^{-1}$ for the *N*-acetyl group of **4**. This observation suggests the possibility of determining *N*-acetyl groups in carbohydrates. Measurement of peak areas for *N*-acetyl groups and anomeric C–H vibrations may lead to molar ratios of *N*-acetyl groups to total glycosides.

*Carboxyl group.* — In the present investigation, two compounds studied contained free carboxyl groups. The carbonyl stretching-vibration of the carboxyl group appears at 1675 and 1745 $cm^{-1}$, respectively, for **3** (see Fig. 1) and **2** (Figure not given) in the solid phase. For D-glucuronic acid, the corresponding band appears[2] at 1707 $cm^{-1}$. Thus, the carboxyl group can be differentiated from the *N*-acetyl group (whose absorption band appears at the lower frequency of 1660–1630 $cm^{-1}$).

In aqueous solution, the band for the carboxyl group of **3** is completely masked by the water band at 1650 $cm^{-1}$ (see Fig. 1). For **2** in water, the corresponding carbonyl stretching-vibration band of the carboxyl group is still visible at 1720 $cm^{-1}$, and the 1648-$cm^{-1}$ band obviously originates from the molecules of water.

These observations again suggest that analysis for a carboxyl group in a carbohydrate must also be conducted with a solid sample, as less-reliable conclusions are obtained with aqueous solutions.

*Hydroxyl group.* — Carbohydrates show OH stretching vibration bands in the region of 3500–3300 $cm^{-1}$. However, it is extremely difficult to correlate all of the OH bands with the structural information, as a complicated network of inter- and intramolecular hydrogen-bonds is involved for many carbohydrates.

An interesting observation was made on examining the OH stretching-vibration of the 5 compounds investigated (see Fig. 4). In the Raman spectra of glycosides containing functional groups that are capable of hydrogen bonding, the OH bands are more complicated than in the spectra of compounds lacking such bonds, and the glycosides containing nonpolar groups have much simpler OH band patterns than those containing polar groups. For instance, both **3** and **2** in the solid state display multiple bands, indicating occurrence of complicated hydrogen-bonding involving OH groups. On the other hand, **1**, **5**, and **4** show only one OH band, at 3534, 3301, and 3488 $cm^{-1}$, respectively. For **4**, there is another band, at 3202 $cm^{-1}$; this originates from the N–H stretching vibration.

*C–H stretching-vibrations.* — All compounds investigated as the solid phase show complicated bands of C–H stretching vibrations in the region of 3000 to 2800 $cm^{-1}$ (see Fig. 5); this may indicate subtle differences between C–H situated at different positions in carbohydrates. In lipids and membranes, modes of Raman bands due to the alkyl C–H stretch are related to the acyl chain-packing structure[14–16].

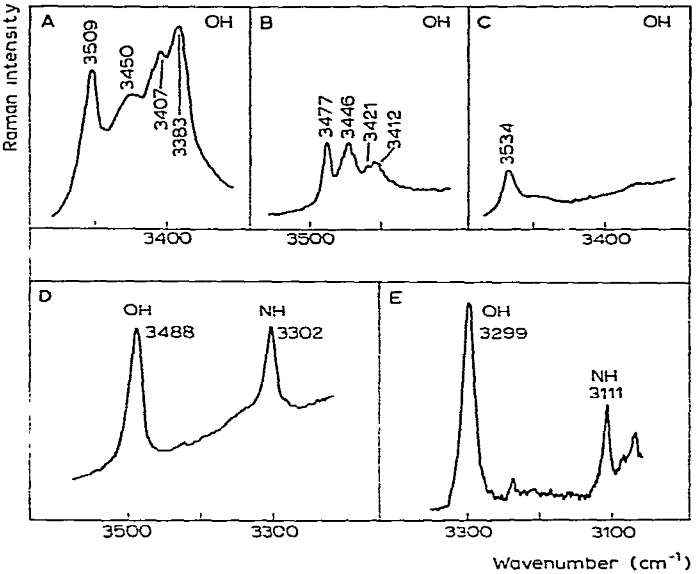


Fig. 4. Laser-Raman spectra of OH vibrational regions of solid samples in the range of 3000 to 2800 $cm^{-1}$. (A, 3, radiant power 300 mW; B, 2, radiant power 400 mW; C, 1, radiant power 300 mW; D, 4, radiant power 300 mW; E, 5, radiant power 200 mW; all at resolution $\pm 5\ cm^{-1}$, integration time 0.2 sec, scanning speed 0.2 $cm^{-1}.sec^{-1}$.)

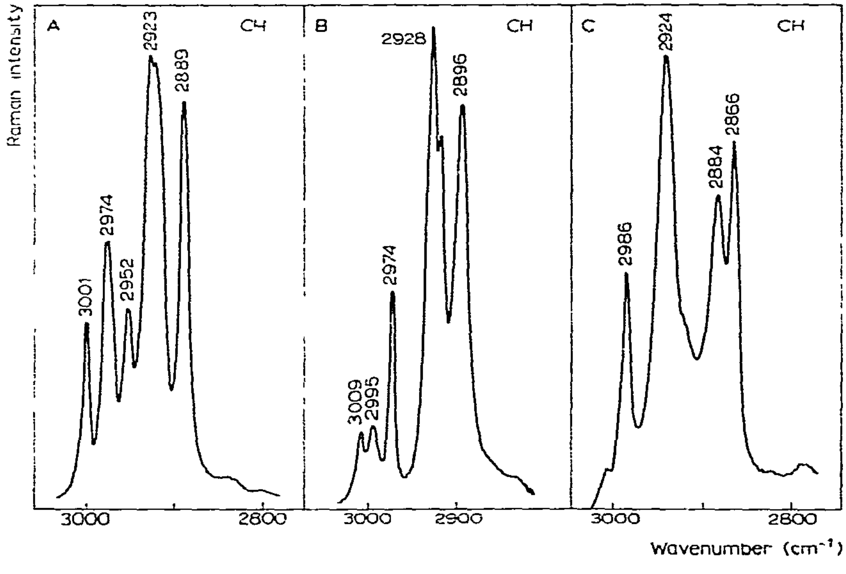


Fig. 5. Laser-Raman spectra of C–H vibrational regions of solid samples in the range of 3500 to 3100 $cm^{-1}$. (A, 3; B, 1; C, 4; all at radiant power 300 mW, resolution $\pm 5\ cm^{-1}$, integration time 0.2 sec, scanning speed 0.2 $cm^{-1}.sec^{-1}$.)

In such simple carbohydrates as those studied here, there is no secondary structure, and thus the C–H stretching-vibration has little significance in this aspect. However, for polysaccharides, where many biological and physical properties of the consituent carbohydrate residue are related to secondary structure, the C–H stretching-vibration region of 3000 to 2800 $cm^{-1}$ may be important.

## ACKNOWLEDGMENTS

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