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STRUCTURE AND SYNTHESIS OF A NOVEL PANTOTHENIC ACID DERIVATIVE, THE MICROBIAL GROWTH FACTOR FROM TOMATO JUICE

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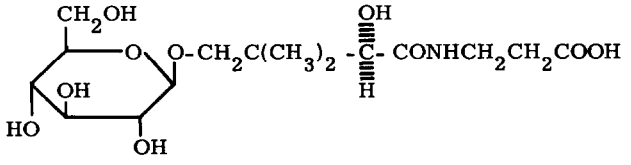
A malo-lactic fermentation*1 bacterium (strain WNB-75)[1] requires essentially some factor contained in tomato and other vegetables or fruits for its growth[2]. The preceding paper reported the isolation procedure of that factor from tomato juice (TJF) and some chemical properties, and suggested that TJF is a β-glucoside containing nitrogenous component[2]. It was also shown that the biological activity of TJF can be substituted by a large amount of D-pantothenate.

In this letter, the structure (I), including its stereochemistry, of TJF is elucidated from NMR spectrum and biological activity of the natural product as compared with those of synthetic materials.



(I)

*1 Bacterial action in wine causing the decrement of acidity by the conversion of malic acid to lactic acid.

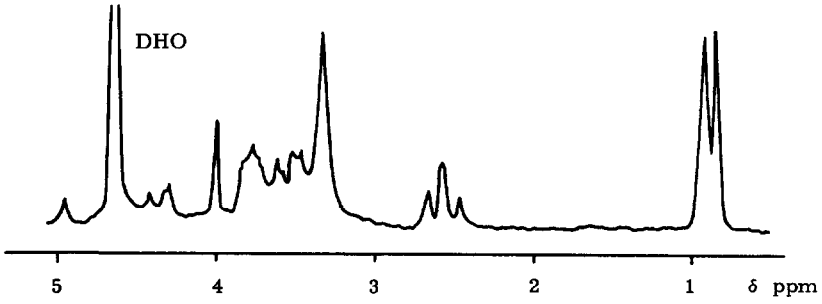


Fig. 1. NMR spectrum of TJF in D_2O.

The NMR spectrum of purified TJF is shown in Fig. 1. Two methyl signals at δ 0.88 and 0.94 ppm are assigned to two tert-methyl groups based on no coupling to other signals. Triplet at δ 2.58 ppm (J=6 cps) is for methylene. The comparison of this spectrum with that of pantothenic acid*2, which can substitute the biological activity for TJF, suggests that the signals (0.88, 0.94 and 2.58 ppm) are derived from the pantothenic acid moiety. The differences of δ values of these spectra are possibly due to the linkage of sugar residue with pantothenic acid in TJF. Doublet signal at 4.38 ppm (J=6 cps) in TJF spectrum is due to the anomeric proton at β-glucosidic linkage[3]. Comparison of the integral value of two methyl groups (0.88 and 0.94 ppm) of pantothenic acid residue with that of δ 3.3 - 3.9 ppm revealed that the ratio of pantothenic acid and glucose moieties in TJF was approximately one to one.

TJF acts as an acid on anion exchange resin column, and pKa' value of TJF (4.20) is close to that of pantothenic acid (4.35). Treatment with diazomethane in methanol and then with pyridine-acetic anhydride (1:1) at -25°C yielded monomethoxy-pentaacetyl derivative of TJF. NMR (in $CDCl_3$, δ ppm): 0.98(6H, s), 2.0 - 2.15(five CH_3CO), 2.54(2H, t, J=6 cps), 3.2 - 5.2 (m), 3.70 (3H, s).

From the results, it was concluded that the probable structure of TJF is that of monoglucosyl pantothenic acid in which a glucosyl residue links to the hydroxyl group at C-2' or C-4' of pantothenic acid moiety. In order to determine the total structure of TJF, further investigation

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*2 NMR (in D_2O, δ ppm) of D-pantothenic acid: 0.89 (3H, s), 0.91 (3H, s), 2.39 (2H, t, J=7 cps), 3.42 (2H, t, J=7 cps), 3.42 (2H, s), 3.96 (1H, s).

was carried out with the synthesis of possible monoglucosyl pantothenic acid derivatives.

The synthesis of 2'-O-(β-D-glucopyranosyl)-DL-pantothenic acid (II) was carried out as follows: a mixture of ethyl 4'-O-acetyl-DL-pantothenate, dry $Hg(CN)_2$ powder and freshly activated calcium sulfate powder in dry nitromethane-benzene (2:1) was stirred for one hour at room temperature. Then tetra-O-acetyl-α-D-glucopyranosyl bromide (AGB) was added to the solution and reaction continued for 20 hours with stirring at boiling temperature of the solvents. The protecting groups from the purified product were removed by hydrolysis with $Ba(OCH_3)_2$ in methanol. The resulting product was identified as II by NMR; (in D_2O, δ ppm): 0.88 (6H, s), 2.58 (2H, t, J=7 cps), 3.1 - 3.8 (m), 3.87 (1H, s), 4.38 (1H, d, J=6 cps).

The synthesis of 4'-O-(β-D-glucopyranosyl)-DL-pantothenic acid (III) was performed by the following procedures: to a mixture of benzyl 2'-O-benzyl-DL-pantothenate, dry $Hg(CN)_2$ powder and freshly activated calcium sulfate powder in dry nitromethane-benzene (2:1), the bromide (AGB) was added and the reaction mixture heated moderately for 10 hours. Purification on silicic acid column gave a desirable condensation product. Hydrogenolysis of the product with palladium black and deacetylation with $Ba(OCH_3)_2$ in methanol yielded a hygroscopic compound, which was assigned as III according to NMR: (in D_2O, δ ppm): 0.88 (3H, s), 0.94 (3H, s), 2.58 (2H, t, J=6 cps), 3.3 - 3.9 (m), 4.03 (1H, s), 4.38 (1H, d, J=6 cps).

4'-O-(β-D-glucopyranosyl)-D-pantothenic acid (IV) was synthesized using benzyl 2'-O-benzyl-D-pantothenate (prepared from D(-) pantolactone) by the same procedure described above. NMR (in D_2O, δ ppm): 0.88 (3H, s), 0.94 (3H, s), 2.58 (2H, t, J=6 cps), 3.3 - 3.9 (m), 4.03 (1H, s), 4.38 (1H, d, J=6 cps).

Biological activities of TJF and synthetic compounds II, III and IV with the bacterium, strain WNB-75, are summarized in Table 1.

NMR spectrum of II is considerably different at methyl region from that of TJF, but III, IV are identical with TJF in spectrometry and chromatography. Biological activity of the DL-form (III) and the D-form (IV) are 50 % and 100 % of TJF respectively as shown in Table 1. Moreover, ORD spectrum of IV almost coinsides with that of TJF. Therefore, the structure of TJF was concluded to be 4'-O-(β-D-glucopyranosyl)-D-pantothenic acid (I).

Table 1.

Compound	NMR of two tert-methyl (D_2O, δ ppm)	Biological activity [a]
TJF	0.88, 0.94	0.05
2'-O-(β-D-glucopyranosyl)-DL-pantothenic acid	0.88	5.0
4'-O-(β-D-glucopyranosyl)-DL-pantothenic acid	0.88, 0.94	0.1
4'-O-(β-D-glucopyranosyl)-D-pantothenic acid	0.88, 0.94	0.05
Calcium-D-pantothenate	0.89, 0.91	5.0

a) The biological activity is shown by respective minimal amount of compound (μg) per ml of medium for adequate growth of strain WNB-75.

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