A Sialic Acid-like Substance Present in Plants. Similarity of Quinic Acid to Sialic Acid in the 2-Thiobarbituric Acid Reaction

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Sialic acid-like substances present in seeds, fruits and various vegetables have been demonstrated exclusively by color reactions.^{1,2)} Fresh fruits of eggplant, peels of summer orange and bamboo shoots are relatively rich in the sialic acid-like substances.³⁾ However, the isolation and structural identification have not been reported.

We now report that quinic acid present widely in plants⁴⁾ behaves like sialic acid in the color reaction with 2-thiobarbituric acid.⁵⁾

Fresh fruits (3.9 kg) of eggplant, *Solanum Melongna* L., were ground in ~4 liters of water. The mixture was adjusted to 0.1 N concentration of H₂SO₄, and the suspension was heated at 80°C for 90 min. The mixture was filtered through cotton sheets, and the filtrate was centrifuged at 3500 rpm for 10 min. The supernatant solution was applied to a column (5 × 50 cm) of IR-120B (H⁺), and the eluate was applied to a column (5 × 60 cm) of Dowex 1 × 8 (borate form). The Dowex column was eluted stepwise with 0.05 to 0.3 N formic acid. The sialic acid-like substance was eluted with 0.15 N formic acid. The fractions showing a positive 2-thiobarbituric acid reaction were combined and concentrated *in vacuo* at ~40°C to afford a syrupy product. Crystallization was performed from a mixture of water, ethanol and ether to afford crude crystals (1.2 g). Two recrystallizations from the same solvent gave a pure sialic acid-like substance, mp 166~169°C, [α]_D²¹ -41.8° (c=1.1, water). IR ν_{max}^{KBr} cm⁻¹: 3530, 3400, 3350, (OH), 2975, 2950 (CH), 1680 (COOH). NMR δ_{TTP}^{D₂O}: 1.5~2.80 (4H, *m*, CH₂), 3.50 (1H, *q*, CH), 3.90 (1H, *q*, CH), 4.10 (1H, *t*, CH). The compound showed a positive 2-thiobarbituric acid reaction,⁵⁾ but negative direct Ehrlich and resorcinol reactions.⁵⁾ In the 2-thiobarbituric acid reaction, a main absorption peak (λ_{max}) appeared at 549 nm, which is the same position as that of sialic acid, together with very weak absorption peaks at 450 nm and 515 nm (shoulder). IR and NMR spectra, and absorption spectra in the 2-thiobarbituric

acid reaction were identical between the isolated sample and an authentic quinic acid [lit.⁶⁾ mp 173~174°C, [α]_D²⁰ -42~-44° (water)]. No depression was observed on admixture with the authentic sample (mp 169~170°C). Anal. Found: C, 43.39; H, 6.47; O, 50.48. Calcd. for C₇H₁₂O₆: C, 43.75; H, 6.29; O, 49.96.

The structure was further confirmed as its peracetate, mp 138~139°C, [α]_D²¹ -22° (c=1.0, ethanol) [lit.⁷⁾ mp 132~136°C, [α]_D²⁰ -22.5° (ethanol)]. IR ν_{max}^{KBr} cm⁻¹: 1750 (C=O in *O*-acetyl), 1700 (COOH), 1240 (C-O in *O*-acetyl). NMR δ_{TTP}^{D₂O}: 1.70~2.80 (16H, 4 × *O*-acetylmethyl and 2 × CH₂), 5.00, 5.35, 5.55 (1H each, CH). The IR and NMR spectra were identical with those of an authentic sample.

Quinic acid was also isolated from peels of summer orange and bamboo shoots by this procedure.

TABLE I. ABSORPTION MAXIMA OF OXIDIZED PRODUCTS OF QUINIC ACID IN THE REACTION WITH 2-THIOBARBITURIC ACID^{a)}

Derivatives of quinic acid	λ _{max} (nm)	
	Main	Minor
2 ^{b)}	549	450 515
3 ^{c)}	532	452
4 ^{d)}	none	none

a) Quinic acid did not produce any color on reaction with 2-thiobarbituric acid.

b) Periodate oxidation product (R=CHO).

c) Periodate oxidation and NaBH₄ reduction product (R=CH₂OH).

d) Citric acid (R=COOH).

R: O=CH- 2
HOCH₂- 3
HOOC- 4

Periodate oxidation of quinic acid (1) was performed in D₂O in an NMR tube (d. 0.4 mm) at 4°C and analyzed by a Hitachi R-24 NMR spectrometer (60 MHz) during the time course of oxidation.⁸⁾ The oxidation was completed in 5 hr to give one mole each of 2 and formic acid {NMR δ_{TTP}^{D₂O}: 8.00 (1H, *s*, HCOO), 5.10, 5.25 (2H, *q*, HC=O), 1.00~2.80 (4H, *m*, 2 × CH₂)}. Upon reaction with 2-thiobarbituric acid, color was formed from 2 (ν_{max}^{H₂O} = 549 nm) and from 3 (ν_{max}^{H₂O} = 532 nm), but not from citric acid (4) (Table I). These data conclude that the color formation of quinic acid in the thiobarbituric acid reaction is due to 3-hydro-2-

hydroxypropionic acid residue produced from quinic acid on periodate oxidation.⁹⁾

REFERENCES

- 1) M. Kanamori, T. Ueda and Y. Kitagawa, *Seikagaku*, **35**, 573 (1963).
 - 2) F. C. Mayer, R. Dam and J. H. Pazur, *Arch. Biochem. Biophys.*, **108**, 356 (1964).
 - 3) K. Onodera, S. Hirano and H. Hayashi, *Agric. Biol. Chem.*, **30**, 1170 (1966).
 - 4) H. G. Fletcher, *Advan. Carbohydr. Chem.*, **3**, 40 (1948).
 - 5) M. W. Whitehouse and F. Zilliken, *Methods Biochem. Anal.*, **8**, 199 (1960).
 - 6) "Data for Biochemical Research," ed. by R. M. C. Dawson, D. C. Elliott, W. H. Elliott and K. M. Jones, Oxford University Press, London, 1969, pp. 96~97.
 - 7) "Beilsteins Handbuch der Organischen Chemie," Vol. 10, 2nd ed., ed. by B. Prager, P. Jacobson, P. Schmidt and D. Stern, Eerlag von Julius Springer, Berlin, 1943, pp. 535~538.
 - 8) S. Hirano, T. Fukuda and M. Sato, *Agric. Biol. Chem.*, **38**, 2537 (1974).
 - 9) "The Amino Sugars," Vol. IA, ed. by R. W. Jeanloz, Academic Press, New York and London, 1969, pp. 231~233.
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