18829-56-6; (*E,Z*)-2,6-nonadienal, 557-48-2; 2-methylbenzaldehyde, 529-20-4; 3-methylbenzaldehyde, 620-23-5; 4-methylbenzaldehyde, 104-87-0; acetophenone, 98-86-2; phenylacetaldehyde, 122-78-1; (*E,Z*)-2,4-nonadienal, 21661-99-4; (*E,E*)-2,4-nonadienal, 5910-87-2; carvone, 99-49-0; butyrophenone, 495-40-9; (*E,Z,Z*)-2,4,6-nonatrienal, 100113-51-7; (*E,E,Z*)-2,4,6-nonatrienal, 100113-52-8; geranylacetone, 3796-70-1; (*E,E,E*)-2,4,6-nonatrienal, 57018-53-8; β-ionone, 79-77-6; 5,6-epoxy-β-ionone, 23267-57-4; methyl heptanoate, 106-73-0; ethyl (*E*)-4-heptenoate, 54340-70-4; [dimethyl phthalate], 131-11-3; benzyl benzoate, 120-51-4; [dibutyl phthalate], 84-74-2; α-terpineol, 98-55-5; myrtenol, 515-00-4; 2-heptylfuran, 3777-71-7; 1-phenoxybutane, 1126-79-0; 1,2-dimethoxybenzene, 91-16-7; benzyl isothiocyanate, 622-78-6; 2-(methylthio)benzothiazole, 615-22-5; 2-butanol, 78-92-2; 2-methyl-3-buten-2-ol, 115-18-4; 1-propanol, 71-23-8; 2-methyl-1-propanol, 78-83-1; 3-pentanol, 584-02-1; 2-pentanol, 6032-29-7; 1-butanol, 71-36-3; 1-peten-3-ol, 616-25-1; 2-methyl-3-heptanol, 18720-62-2; (*Z*)-2-pentenol, 1576-95-0; 1-hexanol, 111-27-3; (*Z*)-3-hexenol, 928-96-1; (*E*)-2-hexenol, 928-95-0; (*Z*)-2-hexenol, 928-94-9; 1-octen-3-ol, 3391-86-4; 2-ethyl-1-hexanol, 104-76-7; 1-octanol, 111-87-5; (*E*)-2-octenol, 18409-17-1; 6-undecanol, 1653-30-1; furfuryl alcohol, 98-00-0; 1-decanol, 112-30-1; 1-phenylethanol, 98-85-1; 2-phenylethanol, 60-12-8; 1-dodecanol, 112-53-8; 2-phenoxyethanol, 122-99-6; 1-tetradecanol, 112-72-1; 1-pentadecanol, 629-76-5; 1-hexadecanol, 36653-82-4; 3-pentanone, 96-22-0; 2-pentanone, 107-87-9; 3-penten-2-one, 625-33-2; 2-methylcyclopenten-1-one, 97665-06-0; (*E,Z*)-3,5-octadien-2-one, 4173-41-5; (*E,E*)-3,5-octadien-2-one, 30086-02-3; 2,6,6-trimethyl-2-hydroxycyclohexanone, 7500-42-7; 2-butanoylfuran, 100113-53-9; 4-methylacetophenone, 122-00-9; 2-pentadecanone, 2345-28-0; (*Z*)-linalool oxide, furanoid, 5989-33-3; (*E*)-linalool oxide, furanoid, 34995-77-2; benzothiazole, 95-16-9; phenol, 108-95-2; 2-ethylphenol, 90-00-6; methyl 9-oxononanoate, 1931-63-1; piperonyl acetate, 326-61-4; 2,3-dimethyl-2-nonen-4-olide, 10547-84-9; dihydroactinidiolide, 17092-92-1.

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Characterization of a Nitrite Scavenger, 3-Hydroxy-2-pyranone, from Chinese Wild Plum Juice

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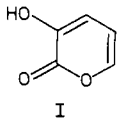
Juice from Chinese wild plum (*Actinidia sinensis*) inhibits nitrosation reactions by efficient scavenging of nitrite. This is due partly to high concentrations of ascorbic acid and partly to a compound not previously reported as a nitrite scavenger, 3-hydroxy-2-pyranone.

Chinese wild plum juice has been traditionally consumed in some districts of China as a preventive for a variety of diseases, including cancer. This fruit is essentially identical with kiwi fruit (both can be designated *Actinidia chinensis* Planch. or *Actinidia sinensis* Planch.), which is known to contain high levels of vitamin C (ascorbic acid) (Selman, 1983). Ascorbic acid is a well-known inhibitor of nitrosation reactions—both in vitro and in vivo (Kamm et al., 1974; Mergens et al., 1980; Mirvish et al., 1972; Mirvish, 1975)—and we have consequently been interested in the inhibitory effects of Chinese wild plum juice on the formation of *N*-nitroso compounds.

In an earlier report (Song et al., 1984), we noted that this juice indeed effectively inhibited the formation of *N*-nitrosomorpholine from morpholine and nitrite and that this effect was due primarily to scavenging of nitrite by constituents of the juice. These properties, in addition, persisted after enzymatic destruction of ascorbic acid, i.e., the juice contained nitrite-scavenging material other than vitamin C. Subsequent experiments (see the Experimental Section) indicated that this additional activity was due to primarily to a single component.

We have isolated this compound from Chinese wild plum juice and now report its identity as 3-hydroxy-2-pyranone (I).



I

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EXPERIMENTAL SECTION

Materials. The Chinese fruit juice was brought by S.P. from the People's Republic of China. Fresh California kiwi fruit was obtained from retail markets in Boston. Reagent

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grade mucic acid (galactaric acid) and potassium bisulfate were purchased from the Aldrich Chemical Co. and were used without further purification.

Sample Preparation. A 50-mL portion of juice was centrifuged at 10000g for 1 h to remove the pulp. The juice (three 10-mL portions) was then placed on Clin-Elut tubes and extracted with 4 volumes of ether. The combined extracts were concentrated to 5 mL with a stream of dry nitrogen and then added to 2.5 mL of 0.01 M ammonium formate at pH 4. The remaining ether was evaporated with dry nitrogen. The buffer solution was then chromatographed by reversed-phase HPLC with 0.01 M ammonium formate as the mobile phase. The eluant was monitored at 254 nm, and each peak was collected and tested for reactivity with nitrite (the eluant between peaks was also tested). A single nitrite-reactive component was detected. The combined active fractions from several runs were saturated with NaCl and extracted with deuteriochloroform. The combined extracts were concentrated with a stream of dry nitrogen to about 0.3 mL and then analyzed directly by 250-MHz ^{1}H NMR and by GC–MS. For experiments with kiwi fruit, the whole fruit was peeled, homogenized in a blender, and then treated in the same manner as the Chinese fruit juice.

Reactions with Nitrite. At room temperature, 10 mL of aqueous ascorbic acid or 3-hydroxy-2-pyranone (6 mM) was added to 10 mL of a solution of sodium nitrite (0.3 mM) at pH 3.3 or 1.65. The buffer was potassium acid phthalate hydrochloride. Solutions of 3-hydroxy-2-pyranone became slightly discolored on standing, so reactions were carried out as soon as possible after preparation of the solutions. The disappearance of nitrite was followed via the automated method described by Green and co-workers (Green et al., 1982). Aliquots (300 μL) were removed at the start of the reaction and every 2 min until approximately 95% of the nitrite had reacted or for at least 1 h for slower reactions. The aliquots were reacted with the Griess reagent (Griess, 1879; Fan and Tannenbaum, 1971), and the nitrite concentration was determined by the absorbance at 540 nm.

Analyses. Gas chromatography–mass spectrometry was done on a Hewlett-Packard Model 5995 GC–MS using a fused-silica capillary column coated with methyl silicone. Helium was the carrier gas for electron ionization experiments, and methane was the carrier gas and reagent gas for chemical ionization experiments. Ultraviolet spectrometry was done on a Hewlett-Packard Model 8450 UV–vis spectrometer interfaced with an HP85 desktop computer.

RESULTS AND DISCUSSION

Proton magnetic resonance analysis of the deuteriochloroform extract of the nitrite-reactive HPLC peak from the juice extract revealed four signals that integrated 1:1:1:1. These consisted of a broad singlet at 6.11 ppm, and three doublets of doublets at 6.18 ppm (J = 5.3, 7.2 Hz), 6.65 ppm (J = 1.6, 7.2 Hz), and 7.14 ppm (J = 1.6, 5.3 Hz). The proton absorbing at 6.11 ppm was exchangeable with deuterium oxide, and the AMX coupling pattern (i.e., three doublets of doublets from three interacting nonequivalent protons; Gunther, 1980) was confirmed by spin decoupling.

Analysis of this same solution by GC–MS with electron ionization (EI) revealed a single major component with signals at m/z 112 (M^+, 83%), 84 (68%), 56 (30%), 55 (100%), 54 (7%), and 53 (10%). The chemical ionization (CI) mass spectrum was dominated by m/z 113 (M + 1, 100%) and also contained minor signals at m/z 141 (M + 29, 7%) and m/z 153 (M + 41, 3%). When an aliquot of this solution was treated with aqueous acidic sodium nitrite and then reinjected, this component had disappeared. These NMR and mass spectral characteristics were consistent with compound I or a close structural analogue. Compound I had been previously reported in some fruit juices (Tatum et al., 1975), and we therefore synthesized authentic 3-hydroxy-2-pyranone, as described by Wiley and Jarboe (1956), via the pyrolysis of mucic acid and potassium bisulfate. The ^{1}H NMR and mass spectra (EI and methane CI) and the GC retention times of this compound were indistinguishable from those of the substance isolated from Chinese wild plum juice. The ^{1}H NMR spectra agree well with published data (Warren et al., 1979; Pirkle and Turner, 1975; Obata et al., 1983; see Table I). The mass spectrum reported by Obata and co-workers (1983) for 3-hydroxy-2-pyranone (m/z 112 (65%), 84 (76%), 56 (42%), 55 (100%), 53 (17%)) is virtually identical with those obtained by us for the compound extracted from juice and for the synthetic compound (see above). The mass spectrum reported by Warren et al. (1979), however, differs from these, most notably in that the base peak occurs at m/z 54 rather than m/z 55. The reasons for this are not known, although instrumental variations in mass spectra are not uncommon; the ^{1}H NMR spectra at any rate leave little doubt that the compound is the same in all cases.

Table I. Proton Magnetic Resonance Spectra of the Chinese Juice Compound and of 3-Hydroxy-2-pyranone

signal	compd from juice extr	synth compd	lit. values[c] ref 1	ref 2
1[a]	6.11	6.13	6.0	6.05
2[b]	6.18	6.20	6.2	6.18
3[b]	6.65	6.66	6.7	6.65
4[b]	7.14	7.15	7.2	7.12

[a] Exchangeable singlet. [b] Doublet of doublets. [c] Reference 1: Wiley and Jarboe, 1956. Reference 2: Warren et al., 1979.

Interestingly, there was no detectable 3-hydroxy-2-pyranone in fresh kiwi fruit purchased from a Boston produce market. As noted above, sufficient amounts of the compound for NMR and GC–MS analysis could be obtained from 30 mL of Chinese juice following concentration to about 0.3 mL. Extraction of 150 mL of kiwi fruit juice, however, gave no indication of 3-hydroxy-2-pyranone even via GC–MS analysis by selected-ion monitoring at m/z 112, 84, and 55. It has been suggested that 3-hydroxy-2-pyranone arises in some cases as a breakdown product of ascorbic acid (Tatum et al., 1969); the relatively high concentrations found in the Chinese juice could presumably have arisen via this route during preparation or storage of the juice.

The reaction of nitrite with 3-hydroxy-2-pyranone was slower than the reaction with ascorbic acid at both pH 3.28 and 1.65. At pH 3.28, approximately 30% of the nitrite had disappeared after 80 min; the reaction with ascorbic acid was about 6 times faster. At pH 1.65, 3-hydroxy-2-pyranone destroyed 95% of the nitrite in about 25 min. At this pH, the reaction of nitrite with ascorbic acid, however, is virtually complete upon mixing. Thus, in view of the large concentrations of ascorbic acid in Chinese wild plum juice (Song et al., 1984), and the higher reactivity of this substance with nitrite, the presence of 3-hydroxy-3-pyranone in this juice, while interesting, is probably of relatively minor significance as an in vivo nitrosation inhibitor. We have, consequently, not further investigated the properties of this compound.

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Registry No. Ascorbic acid, 50-81-7; 3-hydroxy-2-pyranone, 496-64-0; nitrite, 14797-65-0.

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Characterization of 2-*S*-Glutathionylcaftaric Acid and Its Hydrolysis in Relation to Grape Wines

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The major phenolic product formed in grape juice or must by enzymic oxidation is shown to be 2-*S*-glutathionylcaftaric acid by proton NMR studies and fractional hydrolysis. Acidic hydrolysis yields a total of seven new products, the last of which is identical with 2-*S*-cysteinylcaffeic acid synthesized enzymically. All but one of these hydrolysis products were separated by HPLC and identified by comparisons with prepared knowns and by hydrolysis sequence. Aged bottled wines had an increased content of caffeic acid from hydrolysis of caftaric acid and 2-*S*-glutathionylcaffeic acid from hydrolysis of glutathionylcaftaric acid with time. Additional hydrolysis products also appeared to be present in older wines, but uncertainty about the original content, losses to other reactions, and more complicated chromatograms make conclusions uncertain.

Caffeoyl-L-(+)-tartaric (caftaric) acid has considerable interest as the major phenolic substance of grape juice prepared with little oxidation and minimal extraction from skins or seeds. It is also a major nonflavonoid phenol of all freshly fermented grape wines from musts not excessively oxidized (Singleton et al., 1978). In the grape and a few other plants hydroxycinnamoyltartaric acid derivatives apparently replace the chlorogenic (quinic) acid analogues common in most plants. In protected fresh juice from wine grapes about 100 mg/L of *trans*-caftaric acid is considered typical, although there is appreciable varietal variation. About one-fifth as much coutaric (*p*-coumaroyltartaric) acid and a few milligrams/liter of fertaric acid (the ferulic analogue) usually are found (Ong and Nagel, 1978ab; Nagel et al., 1979). Smaller amounts of the cis isomers usually accompany the predominant trans forms of the compounds, and if exposed to light after separation, either isomer is readily returned to an equilibrium mixture of the two (Singleton et al., 1977, 1978). The cis forms have been confused as glucose derivatives, but that has been rectified (Ong and Nagel, 1978a; Baranowski and Nagel, 1981).

In the course of normal commercial juice or must preparation and laboratory sampling without thorough protection from oxidation, a portion of the caftaric and coutaric acids is converted to a new derivative. This derivative is much more polar (in terms of extraction from juice) than the original caftaric acid. The proportion of the new product to the caftaric acid increases, as the exposure to oxygen increases (Singleton et al., 1984). It was shown that this derivative was the result of a four-component reaction involving caftaric (or coutaric) acid, phenoloxidase, oxygen, and glutathione (Singleton et al.,

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