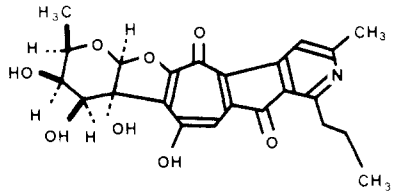
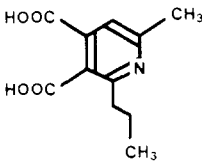
Photooxidative Degradation of Rubrolone in Aqueous Acid Solutions and Its Inhibition by Quercetin-5′-sulfonic Acid

The sunlight irradiation of rubrolone (1) in acid solution (pH 2.8), in the presence of air, yields one major photoproduct that has been characterized as 2-(*n*-propyl)-3,4-dicarboxy-6-methylpyridine (2). This photooxidative bleaching is largely prevented by the presence of quercetin-5′-sulfonic acid (3).

Rubrolone (1) is a red pigment produced by the soil



1



2

actinomycete *Streptomyces echinoruber* (Palleroni et al., 1978), whose structure and stereochemical details have been established by X-ray crystallography (Schüep et al., 1978). Rubrolone holds promise as a food colorant, based on its satisfactory chromatic characteristics, acceptable LD_{50}, and the absence of undesirable biological effects like antibiosis and cytotoxicity so prevalent among microbial metabolites (Palleroni et al., 1978). A 20-ppm solution at pH 2.8 shows an intense burgundy-red color which, however, bleaches rapidly when exposed to sunlight irradiation in the presence of air.

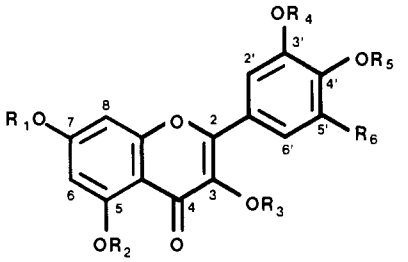
Although hardly uncommon among organic chromophores, such photolability poses limitations to the use of rubrolone in liquid formulations such as carbonated beverages, fruit and vegetable juices, etc.

Based on the ideas advanced in our previous studies on the photostabilization of anthocyanins (Sweeny et al., 1981b) and on the red pigments of *Monascus anka* (Sweeny et al., 1981a), this paper deals with the photobleaching of 1 in acid solution, the chemical nature of its major photoproduct, and the preventive effects exerted by quercetin-5′-sulfonic acid on the photooxidative degradation of 1.

EXPERIMENTAL SECTION

Melting points are uncorrected. ^1H and ^{13}C NMR spectra were run in Me_2SO-d_6 by using a Varian CFT-20 spectrometer. Chemical shifts are given in ppm downfield from DDS. Abbreviations are as follows: m, multiplet; s, singlet; t, triplet. The mass spectra were obtained at 70 eV by using a Du Pont 490 spectrometer. HPLC analyses were conducted on a Waters Instrument Model ALC-GPC-201, equipped with a Waters variable-wavelength detector, Model 450, and a Hewlett-Packard, 3380A integrator–recorder. Rubrolone (1) [λ_{max} (H_2O) 525 nm (log ϵ 3.93)] was kindly supplied by Dr. A. Stempel of Hoffmann-La Roche, Inc., Nutley, NJ. The stability of 1 in 0.01 M citric acid (pH 2.8) was followed spectrophotometrically at 525 nm. Quercetin-5′-sulfonic acid (3) was synthesized as described by Terpilowski et al. (1970). 3′,4′,7-tri(β-hydroxyethyl)rutin (4) was prepared from rutin, following published procedures (Zyma, 1966).

The photoprotection experiments were conducted in 0.01 M citric acid (pH 2.8). Rubrolone (1) was added at 30 ppm and the co-pigments at 100 ppm each. Samples were stored in 100-mL volumetric flasks and exposed to direct sunlight. Fading was followed by reading the absorbance at the λ_{max} (525 nm) as a function of the exposure measured in langleys (cal/cm^2) with an Eppley black and white pyranometer, Model 8-48A, Eppley Laboratory, Inc., Newport, RI. For the latitude of Atlanta, an exposure of 500 langleys is equivalent to a full day of summer sunlight.



3 $R_1 = R_2 = R_3 = R_4 = R_5 = H$; $R_6 = SO_3H$

4 $R_2 = R_6 = H$; $R_1 = R_4 = R_5 = \beta$-hydroxyethyl; R_3 = rutinose

Results are expressed as percent color remaining = $A_{525}(t)/A_{525}(t = 0) \times 100$.

Preparative Photolysis of Rubrolone. A solution of 400 mg of rubrolone in 2 L of 0.01 N HCl was exposed under air to direct sunlight until the bright red color had faded to a light brown (about 5000 langleys of exposure). The solution was then concentrated in vacuo after the addition of 500 mg of sodium acetate. The concentrate (10 mL) was analyzed by HPLC on a 4 × 300 mm C_{18} μBondapak column, using 1% aqueous acetic acid as the eluant and the detector set at 280 nm. The major peak (Figure 1) was then collected by repeated injections on the same instrument. The collected sample was freeze-dried to give 22 mg of 2, a white solid: mp 188–191 °C; UV (H_2O) λ_{max} 284 nm (ϵ 6400); IR (KBr) 3420, 2970, 2930, 2880, 1730, 1660, 1620, 1390, 1245, 1220, 925 cm^{-1}; ^1H NMR δ 0.89 (t, 3 H, J = 7 Hz, $-CH_3$), 1.62 (m, 2 H, $-CH_2-$), 2.46 (s, 3 H, C6-CH_3), 2.74 (t, 2 H, J = 7 Hz, Ar-CH_2-), 7.77 (s, 1 H C5-H); ^{13}C NMR δ 170.65, 168.23, 160.38, 159.46, 139.16, 127.91, 120.92, 38.76, 25.50, 24.08, 15.44.

Treatment of 2 with an excess of CH_2N_2 in MeOH–Et_2O gave the dimethyl ester: MS m/z (rel intensity) 251 (0.26), 236 (31.5), 224 (16.0), 223 (100), 220 (28.7), 208 (30.3), 204 (19.8), 192 (20.6), 135 (28.8), 134 (11.3), 133 (24.0), 132 (18.2), 130 (10.4), 117 (10.0), 108 (12.6), 107 (94.5), 106 (12.9), 93 (32.1).

Nitric Acid Oxidation of Rubrolone. An authentic sample of 2-(*n*-propyl)-3,4-dicarboxy-6-methylpyridine (2) was prepared by oxidation of rubrolone (1.0 g) in 7 mL of concentrated HNO_3 and 1 mL of H_2O, for 1 h at 100 °C (Schüep et al., 1978), and then the mixture was allowed to stand at room temperature overnight. The solution was concentrated to 0.05 mL at reduced pressure (20 mmHg; 100 °C), then 10 mL H_2O was added, and the mixture was again concentrated. A final 10 mL of H_2O was added, and the pH was adjusted to 7 with $KHCO_3$. A 1-mL aliquot was separated by HPLC on a C_{18} μBondapak column using 1% aqeuous acetic acid, to afford 11 mg of 2, a white solid.

0021-8561/81/1429-1292$01.25/0

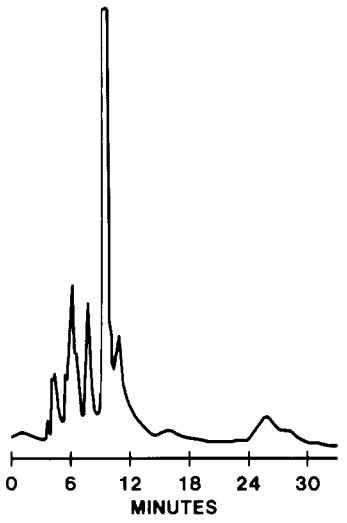


Figure 1. HPLC analysis of the crude rubrolone photolysate. C_{18} μBondapak column; eluant was 1% aqueous AcOH; 1 mL/min.

Table I. Photoprotective Effects of Quercetin-5′-sulfonic Acid (3) and 3′,4′,7-Tri(β-hydroxyethyl)quercetin 3-Rutinoside (4) on the Photooxidative Bleaching of of Rubrolone (1)[a]

	langleys (cal/cm²)				
additive	205	515	814	1060	1317
none (control)	81	53	32	20	13
3	97	90	79	74	70
4	91	72	56	46	38

[a] Results are expressed as percent A_{525nm} remaining relative to 0 langley (for conditions, see Experimental Section).

This was converted to the dimethyl ester with CH_2N_2: MS *m/z* (rel intensity) 251 (0.43), 237 (10.0), 236 (53.2), 224 (24.9), 223 (100), 220 (43.7), 208 (43.1), 204 (31.6), 192 (32.9), 165 (10.6), 164 (13.2), 135 (32.7), 133 (25.5), 132 (23.8), 131 (10.1), 117 (9.8), 108 (10.6), 107 (17.1), 106 (12.5), 93 (26.6).

RESULTS AND DISCUSSION

Our prior finding of the photoprotective effect that quercetin-5′-sulfonic acid (**3**) has on anthocyanins in solution (Sweeny et al., 1981b) prompted us to extend this observation to the photolabile pigment **1**. The results of Table I show that **3** has indeed a remarkable effect on the stability of **1**. Examination of molecular models indicated that **1** can also associate to **3** through π–π, ionic, and H-bonding interactions (Figure 2). This effect is largely lost, as expected, if the phenolic groups of **3** are alkylated and the sulfonic acid group is absent, as is the case for 3′,4′,7-tri(β-hydroxyethyl)quercetin 3-rutinoside (**4**) (Table I). Unlike the anthocyanins, however (Sweeny, et al., 1981b), neither compound produces any shift in the λ_{max} or the extinction coefficient of the pigment.

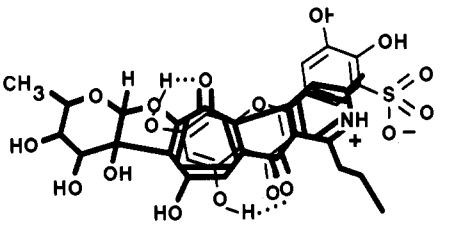


Figure 2. Representation of a 1:1 molecular complex between rubrolone (**1**) and quercetin-5′-sulfonic acid (**3**).

During the course of these studies, it became of interest to identify the photoproduct(s) formed during the bleaching of rubrolone. Direct HPLC analysis of the reaction mixture (see Experimental Section) indicated the presence of a major UV-absorbing (λ_{max} = 284 nm) degradation product (Figure 1).

The product was collected from the column, and its structure was determined as **2** by MS comparisons of its dimethyl ester with that of an authentic sample prepared by the nitric acid oxidation of **1** (Schüep et al., 1978). ^1H and ^{13}C NMR analyses of **2** confirmed its identity.

ACKNOWLEDGMENT

We are grateful to Dr. A. Stempel, Chemical Research Department, Hoffmann-La Roche, Inc., Nutley, NJ, for a generous gift of rubrolone and to Richard Karelitz, The Coca-Cola Company, for the MS measurements.

LITERATURE CITED

Palleroni, N. J.; Reichelt, K. E.; Mueller, D.; Epps, R.; Tabenkin, B.; Bull, D. N.; Schüep, W.; Berger, J. *J. Antibiot.* **1978**, *31*, 1218–1225.

Schüep, W.; Blount, J. F.; Williams, T. H.; Stempel, A. *J. Antibiot.* **1978**, *31*, 1226–1232.

Sweeny, J. G.; Estrada-Valdes, M. C.; Iacobucci, G. A.; Sato, H.; Sakamura, S. *J. Agric. Food Chem.* **1981a**, in press.

Sweeny, J. G.; Wilkinson, M. M.; Iacobucci, G. A. *J. Agric. Food Chem.* **1981b**, *29*, 563–567.

Terpilowski, J.; Manczyk, R.; Kopacz, M. *Diss. Pharm. Pharmacol.* **1970**, *22*, 389–393.

Zyma, S. A. British Patent 1 045 010, Oct 5, 1966.

James G. Sweeny*[1]
Maria C. Estrada-Valdes[1]
Guillermo A. Iacobucci[1]
David J. Goldsmith[2]

[1]Corporate Research and Development Department
The Coca-Cola Company
Atlanta, Georgia 30301
[2]Chemistry Department
Emory University
Atlanta, Georgia 30322

Received for review February 4, 1981. Accepted August 3, 1981.