administered to rats yields less than $^1/_3$ the $^{14}CO_2$ obtained from the first passage through the rat. Adaptation of the intestinal flora to the polysaccharide does not occur. Rats that were prestressed for more than 90 days with polydextrose showed no significant change in metabolic utilization.

As predicted from absorption (persorption) studies of macromolecules, a small amount of intact polydextrose is expected to be absorbed and excreted in urine. Analysis of urinary radioactivity after [^{14}C]polydextrose indicates that a maximum of 0.2% of an orally administered dose is absorbed by the rat.

ACKNOWLEDGMENT

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Photoprotection of the Red Pigments of *Monascus anka* in Aqueous Media by 1,4,6-Trihydroxynaphthalene

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The photobleaching of the *Monascus anka* red pigments *N*-glucosylrubropunctamine (**3**) and *N*-glucosylmonascorubramine (**4**) by sunlight irradiation in aqueous solutions at pH 2.8 and 6.0 is substantially inhibited in the presence of 1,4,6-trihydroxynaphthalene (**10**). This protective effect is specific with respect to the number and position of the hydroxyls in the naphthalene ring and seems related to the ability of **10** to form molecular complexes with **3** and **4**.

Fermentation processes for the production of natural products from microorganisms are central to the needs of the pharmaceutical and food industries in terms of drugs, additives, nutrients, and processing aids (Arima, 1977; Woodruff, 1980).

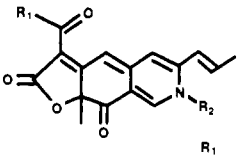
The genetic manipulations of useful microbes through the induction/selection of mutants and the use of DNA recombination techniques (Elander, 1980) will continue to improve the capabilities and efficiency of fermentations.

Against such promising technological background, the available information of the potential of microorganisms as color producers contrasts as fragmentary and superficial. A recent compilation of microbial metabolites (Laskin and Lechevalier, 1973) lists pigmented products whose color, however, is secondary to some significant bioactivity, as in the case of antibiotics, toxins, carcinogens, fungistatics, antitumor compounds, etc. The systematic search for nontoxic, bioinert microbial pigments that could function as food additives is yet to be realized.

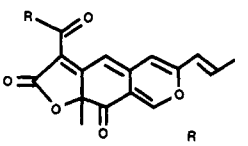
Corporate Research & Development, The Coca-Cola Company, Atlanta, Georgia 30301 (J.G.S., M.C.E.-V., and G.A.I.), and Department of Agricultural Chemistry, Hokkaido University, Sapporo, Japan (H.S. and S.S.).

One important application for food colors is the area of liquid formulations like carbonated beverages, canned fruit juices, and the like. For that end, it is particularly important to establish their colorfastness in solution, as affected by light, pH, and temperature.

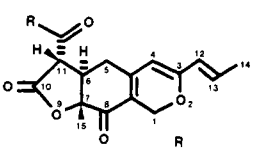
In this paper we discuss our observations on the protection of the red pigments rubropunctamine (**1**) and



	R_1	R_2
1 Rubropunctamine	$n\text{-}C_5H_{11}$	H
2 Monascorubramine	$n\text{-}C_7H_{15}$	H
3 N-glucosylrubropunctamine	$n\text{-}C_5H_{11}$	glucose
4 N-glucosylmonascorubramine	$n\text{-}C_7H_{15}$	glucose



5 Rubropunctatin $n\text{-}C_5H_{11}$
6 Monascorubrin $n\text{-}C_7H_{15}$



7 Monascin $n\text{-}C_5H_{11}$
8 Ankaflavin $n\text{-}C_7H_{15}$

monascorubramine (**2**) against sunlight fading in solution.

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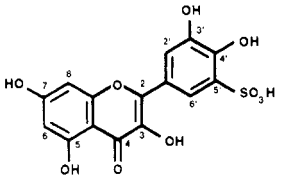
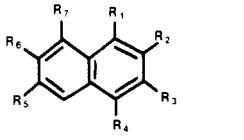
These pigments, produced by the organism *Monascus anka*, are members of the sclerotiorin group of fungal metabolites (Shibata et al., 1964). Whalley (1963) has reviewed the chemistry of this group, and more recently, he has determined the absolute configuration of the two chiral members monascin 7 and ankaflavin 8 (Whalley et al., 1976).

EXPERIMENTAL SECTION

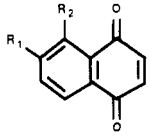
Salicylic acid (Fisher), *p*-aminobenzoic acid (Fisher), 4,8-dihydroxyquinoline-2-carboxylic acid (Aldrich), 5-hydroxy-1,4-naphthoquinone (Aldrich), 4,5-dihydroxynaphthalene-2,7-disulfonic acid potassium salt (Eastman), 1-naphthol-4-sulfonic acid sodium salt (Eastman), and 1-naphthol-2-sulfonic acid potassium salt (Eastman) were used as received from the supplier. Due to the low water solubility of the parent diols, 1,2-, 1,4-, 2,6-, and 2,7-dihydroxynaphthalenesulfonic acids were prepared by sulfonation of the parent dihydroxynaphthalene (1 g) in concentrated H_2SO_4 (6.5 mL) for 30 min at room temperature. Workup consisted of adding the sulfonation mixture to 100 mL of ice–water, neutralizing with solid $CaCO_3$, and filtering out $CaSO_4$. The filtrate was then passed through a Dowex 50W X 4 cation-exchange resin in the Na^+ form and the eluant was freeze-dried. HPLC analysis on a C_{18} μ Bonapak column using HOAc (5%), MeOH (20%), and H_2O (75%) as the eluant showed the products to be mixtures of mono- and disulfonates. These materials were used as such without any further purification. Quercetin-5′-sulfonic acid (**9**) was synthesized as



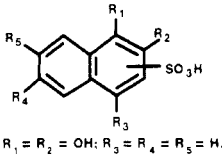
9.



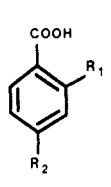
10: $R_1 = R_4 = R_6 = OH$; $R_2 = R_3 = R_5 = R_7 = H$.
17: $R_1 = R_7 = OH$; $R_3 = R_5 = SO_3H$; $R_2 = R_4 = R_6 = H$.
18: $R_1 = OH$; $R_2 = SO_3H$; $R_3 = R_4 = R_5 = R_6 = R_7 = H$.
19: $R_1 = OH$; $R_4 = SO_3H$; $R_2 = R_3 = R_5 = R_6 = R_7 = H$.



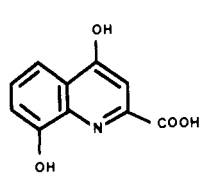
11: $R_1 = OH$; $R_2 = H$
12: $R_2 = OH$; $R_1 = H$



13: $R_1 = R_2 = OH$; $R_3 = R_4 = R_5 = H$.
14: $R_1 = R_3 = OH$; $R_2 = R_4 = R_5 = H$.
15: $R_2 = R_4 = OH$; $R_1 = R_3 = R_5 = H$.
16: $R_2 = R_5 = OH$; $R_1 = R_3 = R_4 = H$.



20: $R_1 = H$; $R_2 = NH_2$
21: $R_1 = OH$; $R_2 = H$



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described by Terpilowski et al. (1970).

Isolation of *Monascus* Pigments from "Red Koji". Extraction and separation of the three types of pigments was conducted by the method described by Hiroi et al. (1975) with some modification. Dried koji cultures of *M. anka* (52.6 kg; Toyo Create Co., Ltd., Niigata, Japan) were successively extracted with *n*-hexane for both yellow and orange pigments and with benzene for the red pigments.

Monascin (7) and Ankaflavin (8) (Yellow Pigments). The *n*-hexane extracts above were concentrated in vacuo, and the residue was crystallized from EtOH, to afford a mixture of yellow and orange crystals. Crystallization from ether gave the less soluble yellow pigments as prisms (6.0 g) which, from the MS data showing two molecular ion peaks at m/z 358 and 386, obviously contained monascin (**7**) mixed with some ankaflavin (**8**). The IR and NMR data were very similar to those reported for monascin in the literature (Inouye et al., 1962; Manchand and Whalley, 1973): mp 142.4–144.1 °C [**7**, 143–145 °C (Inouye et al., 1962)]; *MS* m/z (rel intensity) 386 (1.0), 358 (16.0), 162 (100), 134 (3.7); NMR ($CDCl_3$) δ 0.87 (3 H, t, J = 6.3 Hz, CH_3C—), 1.26 [6 H and 10 H, m, —C-$(CH_2)_3C$— and —$C(CH_2)_5C$—], 1.38 (3 H, s, C15-CH_3), 1.84 (3 H, d, J = 6.8 Hz, C14-CH_3), 2.56 [2 H, m, —$CH_2C(=O)$—], 3.23 (2 H, q, J = 4.8 and 9.0 Hz, C5-H_2), 3.61 (1 H, d, J = 14 Hz, C11-H), 4.65, 4.98 (2 H, q, J = 13.8 Hz, C1-H_2), 5.14 (1 H, s, C4-H), 5.79 (1 H, d, J = 15.6 Hz, C12-H), 6.47 (1 H, dq, J = 15.4 and 6.4 Hz, C13-H); IR (KBr) 2950, 1784, 1716, 1668, 1596 cm^{-1}.

Rubropunctation (5) and Monascorubrin (6) (Orange Pigments). The ether-soluble part resulting from the crystallization of the yellow pigments described above was concentrated to yield crystalline materials which were recrystallized from EtOH as orange plates (12.1 g): mp 140.1–142.2 °C [**6**; 142–143 °C (Hadfield et al., 1967)]. The MS spectrum revealed molecular ion peaks at m/z 354 (**5**) and 382 (**6**). The IR and NMR spectrum were consistent with published data on monascorubrin (Kumasaki et al., 1962): MS m/z (rel intensity) 382 (11.0), 354 (2.4), 298 (100), 256 (47.1), 215 (33.4), 200 (11.1); NMR ($CDCl_3$) δ 0.87 (3 H, t, J = 5 Hz, CH_3C—), 1.34 [10 H and 6 H, m, —$C(CH_2)_5C$— and —$C(CH_2)_3C$—], 1.76 (3 H, s, C15-CH), 2.02 (3 H, d, J = 7.2 Hz, C14-CH_3), 2.98 [2 H, t, J = 7.4 Hz, —$CH_2C(=O)$—], 5.96 (1 H, d, J = 16.4 Hz, C12-H), 6.17 (1 H, s, C5-H), 6.54 (1 H, dq, J = 15.3 and 6.8 Hz, C13-H), 6.88 (1 H, s, C4-H), 7.86 (1 H, s, C1-H); IR (KBr) 2930, 1754, 1728, 1661, 1632 cm^{-1}.

Rubropunctamine (1) and Monascorubramine (2) (Red Pigments). The benzene extract described above was worked up for the isolation of the red pigments. After concentration, acetone was added to give a crude precipitate, which was successively washed with acetone and ether. Recrystallization of the crude pigment from EtOH yielded red needles (13.2 g): mp 206.2–207.4 °C [2; 207–208 °C (Hadfield et al., 1967)]. The MS spectrum revealed two molecular ion peaks at m/z 353 (**1**) and 381 (**2**). These indicated that the pigment crystals were a mixture of two closely related pigments, each one having *n*-caproyl (**1**) and *n*-capryl (**2**) side chains. The IR and NMR spectra were compatible with those published on monascorubramine (Kobayashi et al., 1962; Kumasaki et al., 1962): MS m/z (rel intensity) 381 (0.4), 353 (0.2), 255 (100), 199 (2.4); IR (KBr) 3100, 2920, 1735, 1708, 1659, 1624, 1609 cm^{-1}; NMR ($CCDl_3$) δ 0.89 (3 H, t, J = 6.2 Hz, CH_3—C—), 1.33 [10 H and 6 H, m, —$C(CH_2)_5C$— and —$C(CH_2)_3C$—], 1.91 (3 H, s, C15-CH_3), 2.16 (3 H, d, J = 7 Hz, C14-CH_3), 2.97 [2 H, t, J = 7.0 Hz, —$CCH_2C(=O)$—], 6.31 (1 H, d, J = 13 Hz, C12-H), 6.48 (1 H, s, C4-H), 6.77 (1 H, s, C5-H), 6.83 (1 H, s, C1-H), 7.07 (1 H, dq, J = 13.6 Hz, C13-H).

Preparation of *N*-Glucosylrubropunctamine (3) and *N*-Glucosylmonascorubramine (4). A mixture of 3 + 4 was prepared as described by Moll and Farr (1976), from the purified fraction containing the orange pigments monascorubrin (**6**) and rubropunctatin (**5**). The two products could be readily separated by HPLC analysis on a C_{18} μBondapak column using a Waters liquid chromatograph and a Waters Model 450 detector set at 500 nm. With an eluant of 45% CH_3CN–55% H_2O at a flow rate of 1.0 mL/min, *N*-glucosylrubropunctamine (**3**) had a retention time of 4.9 min and *N*-glucosylmonascorubramine

(4) had a retention time of 7.7 min. In the stability studies these two peaks were analyzed with a Hewlett-Packard 3380A integrator recorder.

Concentrations of 1,4,6-trihydroxynaphthalene (**10**) and 6-hydroxy-1,4-napthoquinone (**11**) were also followed by HPLC on a C_{18} μBondapak column. When a flow rate of 1.0 mL/min and a solvent of HOAc (5%), MeOH (20%), and H_2O were used, **10** and **11** had a retention time of 5.1 and 15.0 min, respectively.

Samples for the sunlight fading experiments were prepared in 0.005 M potassium phosphate buffer, pH 6.0, or 0.01 M citric acid buffer, pH 2.8. The pigment mixture (**3** + **4**) was added so that a solution of $A = 2.0$ at 500 nm was obtained (~75 ppm). The photoprotective agents were used at 100 ppm. Solutions were then stored in Pyrex flasks and exposed to direct sunlight, that was measured in langleys (cal/cm^2) with an Eppley Black and White Pyranometer, Model 8-48A, Eppley Laboratory, Inc., Newport, RI, sensitive to UV, visible, and IR radiation. The rate of fading was determined by measuring the absorbance at the λ_{max} (500 nm) or by following the two pigment peaks by HPLC as described above. In all cases the colors faded to a light brown-yellow color.

Ambient stability studies were conducted at 22 °C in 0.005 M phosphate buffer in the dark under air. Results are expressed as percent color remaining = $[A_{500}(t)/A_{500}(t = 0)] \times 100$.

Although 1,4,6-trihydroxynaphthalene (**10**) and 6-hydroxy-1,4-naphthoquinone (**11**) have both been prepared previously (Fischer and Bauer, 1916), we would like to describe an improved procedure for their synthesis and supply previously unreported spectral data for these materials. The methods were adopted from Fieser and Fieser (1967).

6-Hydroxy-1,4-naphthoquinone (11). To an ice-cold solution of 26.8 g of potassium nitrosodisulfonate [Fremy's salt, freshly prepared as described by Moser and Howie (1968)] in 1.4 L of 0.05 M KH_2PO_4 was added dropwise with stirring and cooling a solution of 7.2 g (0.045 mol) of 1,7-dihydroxynaphthalene (Aldrich) in 200 mL of methanol. After being stirred for 15 min. at 0 °C, the solution was saturated with NaCl and extracted with 200 mL of ethyl acetate. The combined EtOAc layers were dried and evaporated to give a thick brown-orange oil. The oil was chromatographed on a 50 × 4.5 cm silica gel 60 column by using 1% MeOH–$CHCl_3$ as the eluant. Combining the quinone fractions (TLC analysis) followed by hexane trituration gave 3.71 g (0.21 mol, 47%) of 6-hydroxy-1,4-naphthoquinone, recrystallized from EtOAc–hexane: mp 193–196 °C dec. The structure of the product was evident from the spectra data, particularly the NMR, and the correspondence of the MS with a published spectrum (Bowie et al., 1965): NMR (Me_2SO-d_6) δ 7.95 (1 H, d, J = 8.5 Hz, C8-H), 7.45 (1 H, d, J = 2.5 Hz, C5-H), 7.10 (1 H, q, J = 2.5 and 8.5 Hz, C7-H), 6.90 (2 H, s, C2- and C3-H); IR (KBr) 3340, 1650, 1560, 1445, 1370, 1300, 1230, 1135, 1115, 1040, 900, 830, 760 cm^{-1}; UV (MeOH) λ_{max} 252 nm (log ε 4.28), 259 (log ε 4.28), 385 (log ε 3.44); MS m/z (rel intensity) 175 (18.2), 174 (100), 146 (39.0), 120 (59.0), 118 (51.6), 92 (36.1), 90 (10.4), 89 (13.4), 63 (22.7).

1,4,6-Trihydroxynaphthalene (10). A mixture of 1.0 g (5.75 mmol) of 6-hydroxy-1,4-naphthoquinone, 100 mL of ether, 25 mL of H_2O, and 5 g of sodium dithionite was shaken in a separatory funnel for 5 min. An additional 1.0 g of $Na_2S_2O_4$ was added, and the mixture was shaken for 10 more min. The lower layer was separated, and the Et_2O layer was washed with 10 mL of saturated NaCl solution. Drying and evaporating the Et_2O layer gave an off-white solid, 920 mg (5.23 mmol, 91%): mp 159–163 °C dec. The product rapidly turned brown on exposure to air, especially in solution where the quinone **11** was formed as a major product: NMR (MeSO-d_6) δ 8.05 (1 H, d, J = 8.5 Hz, C8-H), 7.40 (1 H, d, J = 2.5 Hz, C5-H), 7.10 (1 H, q, J = 2.5 and 8.5 Hz, C7-H), 6.65 (2 H, AB q, J = 8 Hz, C2-H and C3-H); IR (KBr) 3300, 3200, 1640, 1605, 1560, 1400, 1375, 1330, 1300, 1260, 1210, 1150, 1050, 925, 855, 815, 805, 750 cm^{-1}; UV (MeOH) λ_{max} 218 nm (log ε 4.36), 253 (log ε 4.31), 311 (log ε 3.50), 336 (log ε 3.49); MS m/z (rel intensity) 177 (14.7), 176 (100), 148 (19.6), 147 (38.6), 131 (13.3), 121 (23.0), 120 (27.5), 92 (14.7), 91 (20.0), 65 (16.8), 63 (12.5).

RESULTS AND DISCUSSION

The *M. anka* pigments used in this study were prepared from "red koji" provided by Toyo Create Co., Niigata, Japan. This is usually made by inoculating steamed rice with a culture of *M. anka*, followed by aerobic incubation (koji culture) of the mass until full growth of the mold and then drying and grinding of the fermented materials (Su, 1975). This red powder has been used for centuries for the coloration of rice wine (China; Japan) and soybean curds (Korea; Okinawa). Current production of red koji in Japan has reached 100 tons/year and it is found novel food applications, like the coloration of processed meats (sausages; hams), marine products like kamaboko (fish paste) and tarako (cod's roe), and tomato ketchup (Yamanaka, 1977).

The red koji available to us was found to contain, besides the major red pigments **1** and **2**, two orange (**5** and **6**) and two yellow (**7** and **8**) ones, as known from previous work (Whalley, 1963). The fractionation procedure adopted in this work (see Experimental Section) allowed the separation of the three structural types, each one as a mixture of the *n*-caproyl and *n*-capryl side chain analogues. Although a prepared mixture of the six was resolvable by HPLC, this step was not used in the preparative runs.

Because of the poor water solubility exhibited by **1** and **2**, the *N*-glucosyl derivatives **3** and **4** were prepared by a published procedure (Moll and Farr, 1976) and used as such in this study. All photochemical experiments were done in 0.01 M citric acid, pH 2.8, or in phosphate buffer, pH 6.0. These pH ranges were considered adequate for the most commonly envisioned food applications. The derivatives **3** and **4** exhibited a measurable rate of nonphotochemical (solvolytic?) bleaching in solution that did not vary significantly between pH 3.0 and 6.0 pH (Figure 1). This background fading affected the measured photochemical rates by no more than 15%, as the irradiations were completed within 48 h.

Our findings on the photoprotective effect that quercetin-5′-sulfonic acid (**9**) has on anthocyanins in solution (Sweeny et al., 1981) prompted us to test this compound first with the photolabile pigments **3** and **4**. An irradiation experiment done with **9** and a mixture of **3** + **4** at pH 2.8, however, gave negative results (Table I). As a molecular fit between **3** (or **4**) and **9** was not evident from models, as seen for anthocyanins, it was concluded that the ability to associate and the manifestation of photoprotective effects in dilute aqueous solutions should be related phenomena. The influence of molecular structure on exciplex formation and on energy transfer processes in photoexcited systems has been reviewed recently (Mataga and Ottolenghi, 1979).

Adopting those ideas as a working hypothesis, and considering the molecular dimensions of **4**, it was inferred from the examination of molecular models that the simple compound 1,4,6-trihydroxynaphthalene (**10**) could provide

Table I. Effect of Phenolic Additives (100 ppm) on the Bleaching of a Mixture of *N*-Glucosylrubropunctamine (3) and *N*-Glucosylmonascorubramine (4) upon Sunlight Irradiation, in 0.01 M Citric Acid, pH 2.8[a]

	langleys (cal/cm²)					
additives (100 ppm)	100	200	300	420	575	800
none (control)	78	59	41	25	10	2
quercetin-5′-sulfonic acid (9)	87	72	57	44	29	15
1,4,6-trihydroxynaphthalene (10)	96	89	81	75	66	51
1,2-dihydroxynaphthalenesulfonic acid (13)	85	68	51	37	21	8
1,4-dihydroxynaphthalenesulfonic acid (14)	72	52	37	23	11	5
2,6-dihydroxynaphthalenesulfonic acid (15)	53	16	1			
2,7-dihydroxynaphthalenesulfonic acid (16)	60	27	7	2		
4,5-dihydroxynaphthalene-2,7-disulfonic acid (17)	55	32	21[b]	17[b]	16[b]	19[b]
1-naphthol-2-sulfonic acid (18)	69	45	30	15	8	8
1-naphthol-4-sulfonic acid (19)	75	49	29	11	3	
p-aminobenzoic acid (20)	76	56	39	21	7	2
salicylic acid (21)	74	51	31	11	1	

[a] Values are percent color remaining. [b] Yellow-brown color formed.

Table II. Effect of Phenolic Additives (100 ppm) on the Bleaching of a Mixture of *N*-Glucosylrubropunctamine (3) and *N*-Glucosylmonascorubramine (4) upon Sunlight Irradiation, in 0.005 M Phosphate Buffer, pH 6.0[a]

	langleys (cal/cm²)					
additives (100 ppm)	100	200	300	400	600	800
none (control)	81.3	71.6	53.8	37.6	22.4	15.2
1,4,6-trihydroxynaphthalene (10)	96.8	94.4	87.7	82.2	77.1	72.4
6-hydroxy-1,4-naphthoquinone (11)	92.2	89.7	82.3	74.9	70.4	85.8
5-hydroxy-1,4-naphthoquinone (12)	85.9	79.4	64.8	55.5	45.9	37.2
1,2-dihydroxynaphthalenesulfonic acid (13)	86.0	78.5	70.0	61.0	42.5	32.5
1,4-dihydroxynaphthalenesulfonic acid (14)	81.0	68.7	57.5	48.2	36.0	17.5
2,6-dihydroxynaphthalenesulfonic acid (15)	60.7		31.4	17.9		
2,7-dihydroxynaphthalenesulfonic acid (16)	68.3		44.9	31.5		
4,5-dihydroxynaphthalene-2,7-disulfonic acid (17)	81.1	73.8	60.8	36.9	27.1	23.4
1-naphthol-2-sulfonic acid (18)	76.9	68.1	55.9	46.7	29.3	22.1
1-naphthol-4-sulfonic acid (19)	76.9	68.2	54.0	44.6	24.3	15.1
p-aminobenzoic acid (20)	80.9	70.6	56.1	46.0	28.4	18.8
salicylic acid (21)	61.4	46.8	31.0	20.1	7.2	3.7
4,8-dihydroxyquinoline-2-carboxylic acid (22)	85.9	76.8	65.2	54.5	36.5	25.1

[a] Results are expressed as percent of remaining A_{500} with respect to zero langley exposure.

Table III. Photoprotection of *N*-Glucosylrubropunctamine (3) and *N*-Glucosylmonascorubramine (4) by 1,4,6-Trihydroxynaphthalene (10; 100 ppm) and 6-Hydroxy-1,4-naphthoquinone (11; 100 ppm) in 0.005 M Phosphate Buffer, pH 6.0, As Determined by HPLC Analysis (See Experimental Section)

		langleys (cal/cm²)					
pigment	additive	100	200	300	400	600	700
3	none	71.8	61.1	42.7	26.6	<5	
	+10	84.7	78.9	71.6	51.8	48.6	32.4
	+11	82.4	78.3		51.4	50.2	31.4
4	none	71.4	58.9	39.6	21.8	<5	
	+10	86.5	82.2	73.7	58.7	50.0	30.1
	+11	80.8	73.4		45.0	43.1	26.2

the necessary structural elements for (a) molecular juxtaposition between the planar, aromatic moieties of both partners and (b) the formation of three H bonds, involving the unpaired electrons of the nitrogen and the two carbonyls of 4 as proton acceptors (Figure 2).

As shown in Table I, the experimental results confirmed this inference. As is clearly indicated, only 10, out of the ten related phenolic compounds tested, showed a significant photoprotective effect, although all the compounds have similar UV spectral characteristics. None of the compounds showed any effect on the λ_{max} or extinction coefficients of 3 and 4.

Similar results were observed in phosphate buffer, pH 6.0 (Table II). In addition to 10, the corresponding quinone 11 was found equally photoprotective towards 3 + 4 and twice as effective as the isomeric quinone 12. In fact, under the photooxidative conditions of the experiment, it could be shown by HPLC that 10 was totally converted into 11 after 200 langleys, about 25% of the usual irradiation exposure adopted in these studies. The

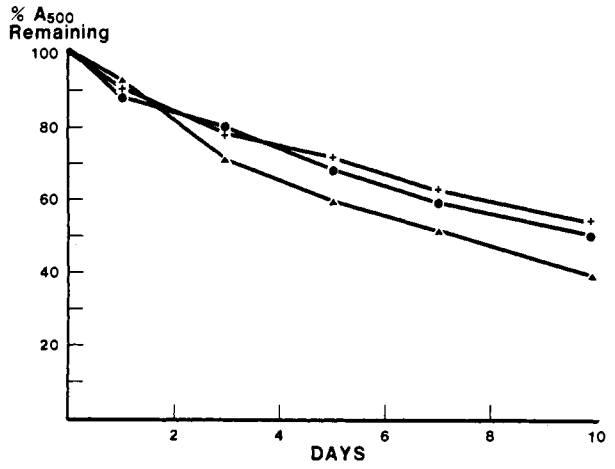

Figure 1. Effect of pH on the stability of the *N*-glucosylrubropunctamine (3) and *N*-glucosylmonascorubramine (4) mixture. (▲) pH 3.0; (●) pH 5.0; (+) pH 6.0.

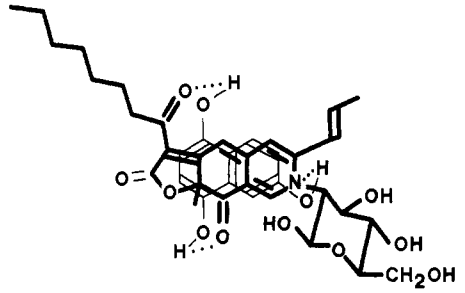

Figure 2. Representation of a (1:1) molecular complex between *N*-glucosylmonascorubramine (4) and 1,4,6-trihydroxynaphthalene (10).

quinones 11 and 12 were not tested at pH 2.8 (Table I) because of their negligible solubility in diluted aqueous acids.

The remarkable photoprotective effects shown by 10 and 11 on the individual red pigments 3 and 4 were confirmed by following the reaction by HPLC, under conditions (see Experimental Section) that fully resolved the mixture 3 + 4. The rate of fading was then determined by area integration of the individual peaks (Table III). As before, the significant protective effects of 10 and 11 were clearly evidenced.

In summary, these observations with the red pigments of *M. anka* emphasize further the importance that molecular association has in photointeractive processes among chemical species in solution, a phenomenon already seen (Sweeny et al., 1981) in the photoprotection of anthocyanins by quercetin-5′-sulfonic acid.

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