however, were not as rapid as those from the 16% (w/w) devitrified dispersion.

It is doubtful whether the increased solubility of primidone in the citric acid contained in the diffusion layer surrounding the dissolving devitrified dispersion could account for the differences in dissolution of the physical mixture and the devitrified dispersion. If it is assumed that the diffusion layer thickness is about 3×10^{-3} cm (7) and that the diffusion layer is saturated with citric acid, then calculations indicate that the maximum amount of primidone that could be in solution is 1–10% of the primidone content of the system (depending upon the primidone concentration in the dispersion). Thus, a gross excess of primidone is present in all cases.

The precipitated primidone crystals were very cohesive, and incomplete wetting could have inhibited dissolution from the physical mixtures. To test this possibility, the dissolution experiment was repeated using another 200-mg sample of the 16% (w/w) physical mixture of precipitated primidone and citric acid previously dispersed with 0.4 ml of polyethylene glycol 300[6]. A sample of the 16% (w/w) devitrified dispersion was also examined in the same manner. From the results (Fig. 4), it can be seen that the dissolution of the devitrified dispersion was not significantly affected by the addition of the polyethylene glycol. The dissolution of the physical mixture was, however, increased and was greater than that of the devitrified dispersion, even though complete dispersal of the mixture was not achieved.

These results indicate that the primidone particles precipitated during dissolution of the devitrified system remain as loose agglomerates during the dissolution process. The collection of these particles and their redispersal with polyethylene glycol in the mixture with citric acid probably result in the destruction of these agglomerates to produce individual particles. The resulting increase in the primidone surface area is responsible for the higher dissolution rate seen with these physical mixtures.

The enhanced dissolution rate of primidone in these devitrified systems (2) is thus confirmed to be due to the reduction in particle size of the primidone in the system and the improved wetting characteristics of the solid dispersion system. Nevertheless, the dissolution rate is impaired by the failure of the precipitated primidone particles to separate completely during dissolution. The enhanced dissolution rates of the 3 and 8% (w/w) devitrified glasses could be due to better particle separation during dissolution.

[6] Koch Light Laboratories, Colnbrook, Bucks, United Kingdom.

CONCLUSIONS

Interactions between primidone and citric acid occur when they are fused together, and viscous supercooled liquids are produced when the melt is cooled. The viscosity of the supercooled liquids increases with increasing primidone concentration, as reflected in an increased glass transition temperature.

During dissolution of the devitrified systems, primidone is precipitated in aggregates of fine particles. The relatively large surface area produced by this process results in the powdered devitrified dispersions (150–250-μm size) having a faster rate of dissolution than physical mixtures of primidone and citric acid of the same size.

The aggregation of primidone particles during dissolution appears to be dependent on the primidone concentration in the dispersion. At low concentrations (3% w/w), the aggregation is less than at 8–16% (w/w), resulting in a faster dissolution of primidone from the devitrified dispersion.

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Phytochemical Investigation of Xanthones of *Eustoma grandiflorum* (Raf.) Shinners

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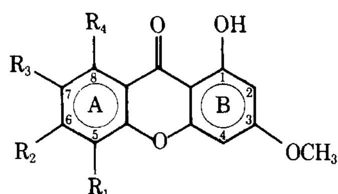
Abstract □ Six polyoxygenated xanthones were isolated from the roots of *Eustoma grandiflorum* (Raf.) Shinners collected in Texas. Structural elucidation of five of these xanthones (1-hydroxy-3,7-dimethoxyxanthone, 1-hydroxy-3,5-dimethoxyxanthone, 1-hydroxy-3,5,6,7-tetramethoxyxanthone, 1-hydroxy-3,5,6,7,8-pentamethoxyxanthone, and 1-hydroxy-3,7,8-trimethoxyxanthone) was accomplished *via* UV, IR, NMR, and mass spectrometry; traditional physical-chemical methods; or direct comparison with a prepared derivative. The sixth xanthone derivative was characterized incompletely. The pentamethoxyxanthone is a new compound and has been designated generically as eustomin. The probable occurrence of these six xanthone compounds in the plant as glycosides is reported.

Keyphrases □ *Eustoma grandiflorum*—methanol extract of roots, six polyoxygenated xanthones isolated □ Xanthones, polyoxygenated—six isolated from methanol extract of roots of *Eustoma grandiflorum* □ Hydroxyxanthones—six isolated from methanol extract of roots of *Eustoma grandiflorum*

Eustoma grandiflorum (Raf.) Shinners (Gentianaceae), a plant primarily indigenous to the southwestern portion of the United States, is commonly referred to as the catchfly-gentian, Texas bluebell, and lira de San Pedro. It has a very showy, eye-catching flower, blooming in great profusion in several areas of Texas. A prominent taproot is evident, and even a cursory examination reveals a bright yellow pigment.

The Gentianaceae family is known to produce xanthone derivatives liberally, and several have been isolated from related genera such as *Canscora, Frasera, Gentiana,* and *Swertia* (1–5). Plant extracts obtained from these genera



I: $R_1 = R_2 = R_4 = H, R_3 = OCH_3$
II: $R_1 = OCH_3, R_2 = R_3 = R_4 = H$
III: $R_1 = R_2 = R_3 = OCH_3, R_4 = H$
IV: $R_1 = R_2 = R_3 = R_4 = OCH_3$
V: $R_1 = R_2 = H, R_3 = R_4 = OCH_3$

are used to treat various ailments including constipation, nervous debility, tuberculosis, fever, and anorexia. Since certain chemotaxonomic relationships often occur among related genera and since results of a preliminary TLC examination of the root extract were promising, it was postulated that xanthones might be present in *E. grandiflorum*.

EXPERIMENTAL

Instrumentation—All NMR spectra[1] were determined in deuterated chloroform solutions in microcells unless otherwise stated. Tetramethylsilane served as an internal standard. IR spectra[2] were determined in potassium bromide pellets. Only significant bands are quoted. All molecular weights were obtained by mass spectrometry[3]. UV spectra were determined with a double-beam spectrophotometer[4] equipped with a recorder[5]. Determinations were made in 95% ethanol unless otherwise stated.

Melting points were determined on a Koffler hot-stage microscope and are uncorrected. Sublimation was performed in a sublimation tube fitted with a cold-finger condenser under vacuum and heat. Silicone fluid[6] was used as the heating medium. Liquid chromatography[7] was performed with Porasil A (37–75 μm) as the column packing and chloroform–methanol (5:1) as the mobile phase.

Plant Material—The plant material was collected near Brenham, Washington County, Tex., in late August of 1971[8]. The air-dried roots (705 g) were ground to a coarse powder in a Wiley mill and subjected to continuous soxhlet extraction with methanol for 60 hr.

TLC—Several investigators (1–5) used TLC procedures to separate xanthones, but none of these procedures was satisfactory for this investigation. Silica gel G plates, prepared according to Stahl (6) and developed in benzene–ethyl acetate–acetic acid (90:5:5), resulted in excellent separation of xanthone constituents. Examination of the methanol extract by this procedure indicated the possible presence of five xanthone constituents. These constituents were detected visually as discrete yellow spots or by UV light whereby the spots fluoresced a rust-red color. This system was employed in all preparative work unless otherwise stated.

Hydrolysis of Root Extract—The methanol was removed under reduced pressure, and the resulting dark-reddish-black extract was treated with four 500-ml portions of 3% aqueous hydrochloric acid. Each portion was boiled over steam for 1 hr, and finally all portions were combined and allowed to cool.

Partition of Hydrolyzed Root Extract—The cooled hydrolyzed root extract was partitioned with benzene. Initial partitions were deep red in color, but repeated partitionings were carried out until a colorless benzene fraction was obtained. The aqueous portions were discarded. The benzene portions were combined, filtered, and allowed to stand at room temperature for several hours. A lemon-yellow compound precipitated from the pooled benzene fractions and was removed by gravity filtration.

Isolation of Xanthone II—The lemon-yellow precipitate from the benzene fractions was purified by column chromatography[9], employing dichloromethane as the eluting solvent. It was crystallized from methanol as yellow needles and designated Xanthone II.

Benzene Partitions—The fraction containing benzene partitions from which Xanthone II had precipitated was placed on a chromatographic column and eluted with dichloromethane. Three major fractions, A-1, A-2, and A-3, were collected.

Xanthone I from Fraction A-1—Fraction A-1 was subjected to high-pressure liquid chromatography with a mobile phase of chloroform–methanol (5:1). One yellow compound was obtained and further purified by preparative TLC. The compound was recrystallized as fluffy, pale-yellow needles from methanol and designated Xanthone I.

Xanthone III from Fraction A-2—Preparative TLC of Fraction A-2, using benzene–ethyl acetate–acetic acid (90:5:5), yielded a yellow compound. Upon recrystallization from methanol, this compound was crystallized as pale-yellow needles and labeled Xanthone III.

Xanthones IV and V from Fraction A-3a—Fraction A-3 was placed on a column and again eluted with dichloromethane, resulting in the collection of two distinct fractions, A-3a and A-3b. Fraction A-3a was subjected to preparative TLC with carbon tetrachloride–methanol (25:1) as the solvent system. Two compounds, Xanthones IV and V, were obtained as pale-yellow needles from methanol.

Xanthone VI from Fraction A-3b—This fraction was evaporated to dryness and sublimed under reduced pressure. This procedure yielded a small amount of yellow material, which was recrystallized from methanol as yellow needles and designated Xanthone VI.

RESULTS AND DISCUSSION

Preliminary examination of the methanol extract of the root material of *E. grandiflorum* by TLC initially revealed the possible presence of five xanthone constituents. These constituents exhibited R_f values of 0.93, 0.86, 0.79, 0.63, and 0.49. It was later shown that six xanthone derivatives had actually been isolated, since two compounds exhibited identical migration patterns and resided at R_f 0.63. These two xanthones were easily separated by TLC procedures with a solvent system of carbon tetrachloride–methanol (25:1). These compounds then exhibited R_f values of 0.73 and 0.65.

The structural characterization of the isolated and purified compounds is discussed in the order in which they were isolated.

1-Hydroxy-3,5-dimethoxyxanthone (II)—This compound exhibited an R_f value of 0.86 and a melting point of 174°. Its UV absorption spectrum was characteristic of a 1,3,5-trioxygenated xanthone (7). Mass spectrometry revealed the parent ion and base peak to be m/e 272, corresponding to a xanthone derivative containing one hydroxyl and two methoxyl functions. The compound exhibited positive ferric chloride (8) and Wilson's boric acid (9) tests and was insoluble in aqueous sodium carbonate (1)[10].

These chemical and physical data indicated the presence of a free phenolic hydroxyl *ortho* to a carbonyl and also a methoxyl group at C-3 on the xanthone nucleus. Further confirmation of the hydroxyl function *ortho* to a carbonyl was accomplished utilizing IR spectrometry, since a strong band due to carbonyl absorption occurred near 1650 cm^{-1} (7, 8). Xanthone II exhibited a significant band at 1650 cm^{-1}. Final elucidation was accomplished utilizing NMR spectroscopy. Two singlets representing six protons at 6.17 and 6.03 τ accounted for the presence of two methyl group protons. From previous NMR studies, it was shown that a xanthone having substituents at C-1 and C-3 exhibited characteristic signals. In general, a pair of doublets occurred in the 3.2–3.7 τ region. The proton on C-2 always occurred at a higher field than the proton on C-4.

Furthermore, a signal far downfield may be attributed to a hydroxyl proton on C-1 (or the equivalent C-8 position) due to a deshielding effect of the adjacent carbonyl (2, 10). Thus, two doublets at 3.68 and 3.48 τ represented two aromatic protons, one at C-2 and the other at C-4. A signal far downfield at −2.31 τ represented a hydroxyl proton on C-1. Carbons 1 and 3 must be occupied by a hydroxyl and a methoxyl function, respectively. Since all positions on ring B were accounted for, only ring

[1] Japanese Electron Optics Lab. model C-60 HL or Varian A-60 spectrometer.
[2] Beckman IR 5A.
[3] DuPont Instruments model 214-91 spectrometer.
[4] Perkin-Elmer model 124.
[5] Rikka Denki model B-161.
[6] Dow Corning DC550.
[7] Waters Associates model ALC/GPC-202 liquid chromatograph.
[8] The plant material utilized was identified as *Eustoma grandiflorum* (Raf.) Shinners (Gentianaceae) by Dr. B. L. Turner, Department of Botany, University of Texas at Austin. A voucher specimen [Sullivan-S. N. (Acc. No. 298773)] representing material collected for this investigation is available for inspection at the Herbarium, Department of Botany, University of Texas at Austin.

[9] All separations by column chromatography were carried out by using 100–200-mesh Bio Sil A silicic acid.
[10] All test reagents were prepared immediately prior to use.

A could contain the remaining methoxyl function. A quartet at 2.20 τ was indicative of a proton on C-8 splitting with two protons exhibiting a multiplet at 2.68–3.03 τ on C-6 and C-7 (2). Thus, the second methoxyl group was placed at C-5.

NMR: −2.31 (s, OH), 3.68 (d, 2-H), 3.48 (d, 4-H), 2.68–3.03 (m, 6,7-H), 2.20 (q, 8-H), 6.17 (s, 3,5-OCH_3), and 6.03 (s) ppm [lit. (11) NMR ($CDCl_3$): 3.69 (d, 2-H), 3.51 (d, 4-H), 2.65–3.00 (m, 6,7-H), and 2.24 (q, 8H) ppm]; UV: λ_{max} (ethanol) 354 (log ϵ 3.53), 307 (4.10), and 244 (4.49) nm [lit. (11) UV: λ_{max} (ethanol) 355 (log ϵ 3.75), 306 (4.28), and 245 (4.60) nm; (1) 308 (4.27) and 245 (4.60) nm; (12) 355 (3.66), 335 sh (3.70), 310 (4.24), and 245 (4.59) nm]; mp 174° [lit. (1) mp 173–174°; (11) 174–175°; (12) 170–171°]; IR_{max}: 1650, 1613, and 1577 cm^{-1} [lit. (11) IR (mineral oil mull): 1662, 1610, and 1575 cm^{-1}].

1-Hydroxy-3,7-dimethoxyxanthone (I)—The second compound isolated and purified exhibited an R_f value of 0.93 and a melting point of 168–169°. Mass spectrometry revealed the parent ion and base peak to be *m/e* 272. This mass again corresponds to a xanthone derivative having one hydroxyl function and two methoxyl groups. It exhibited positive Wilson's boric acid and ferric chloride tests and was insoluble in aqueous sodium carbonate. These data similarly pointed to a compound having a free phenolic hydroxyl *ortho* to a carbonyl and the presence of a methoxyl group at C-3. IR spectrometry verified the presence of an *ortho*-hydroxyl, since a major band of absorption occurred at 1645 cm^{-1}.

By utilizing NMR spectroscopy, final structural elucidation was accomplished. Two singlets representing six protons at 6.10 and 6.11 τ corresponded to two methyl group protons, while two doublets at 3.69 and 3.60 τ occurred due to a proton at C-2 and C-4, respectively. A low field signal at −2.92 τ indicated the presence of a hydroxyl proton on C-1. These assignments placed a hydroxyl and a methoxyl function on ring B, thus leaving final placement of the second methoxyl on ring A. A multiplet at 2.68 τ was attributed to single protons on C-5 and C-6, while a quartet at 2.40 τ arose from a proton splitting with those on C-5 and C-6 and was assigned to C-8. Thus, the second methoxyl substituent must be located at C-7.

NMR: −2.92 (s, OH), 3.69 (d, 2-H), 3.60 (d, 4-H), 2.68 (m, 5,6-H), 2.40 (q, 8-H), 6.10 (s, 3,7-OCH_3), and 6.11 (s) ppm [lit. (2) NMR ($CDCl_3$): −2.94 (s, OH), 3.70 (d, 2-H), 3.58 (d, 4-H), 2.68 (m, 5,6-H), and 2.41 (q, 8H) ppm]; UV: λ_{max} (ethanol) 368 (log ϵ 3.76), 307 (4.12), 260 (4.58), and 232 (4.49) nm [lit. (13) UV: λ_{max} (ethanol) 311 (log ϵ 4.2), 261 (4.7), and 239 (4.5) nm; (14) (methanol) 369 (3.83), 292 (4.39), 259 (4.59), and 231 (4.54) nm; (2) (ethanol) 396 (4.80), 308 (4.14), 259 (4.59), and 238 (4.46) nm]; mp 168–169° [lit. (2) mp 167.5–169°; (15) 167°]; IR_{max}: 1645, 1608, and 1575 cm^{-1}.

As further proof of structure, a synthesis of I (methylgentisin) was accomplished by selective methylation of commercially available gentisin[11] (1,7-dihydroxy-3-methoxyxanthone) (11). Simultaneous development of I with methylgentisin on a TLC plate gave identical spots having an R_f value of 0.93. Chemical and physical test results were identical to those obtained for I. Furthermore, spectral data were also almost identical to those obtained for I.

NMR ($CDCl_3$): −2.94 (s, OH), 3.70 (d, 2-H), 3.58 (d, 4-H), 2.68 (m, 5,6-H), 2.41 (q, 8-H), 6.08 (s, 3,7-OCH_3), and 6.11 (s) ppm; UV: λ_{max} 234, 258, 306, and 368 nm; mp 168°; IR_{max}: 1645, 1610, and 1577 cm^{-1}.

1-Hydroxy-3,5,6,7-tetramethoxyxanthone (III)—Xanthone III exhibited an R_f value of 0.79 and a melting point of 178°. Mass spectrometry indicated a parent ion and base peak at *m/e* 332, corresponding to a xanthone compound having one hydroxyl group and four methoxyl functions. Chemical and physical test results were identical to those mentioned for the previous compounds, and an IR analysis showed a major band at 1653 cm^{-1}. NMR data indicated three singlets at 5.95, 6.04, and 6.15 τ, which were assigned to four methyl group protons. Two doublets at 3.64 and 3.47 τ were assigned to protons on C-2 and C-4, respectively.

From the preceding data, it could be ascertained that all positions on ring B were fully accounted for, with one hydroxyl group on C-1 and a methoxyl group on C-3. Therefore, the three methoxyl functions left to be assigned could only be on ring A. With three of four positions on ring A being occupied, only one singlet for an aromatic proton would be expected; indeed, this signal was observed at 2.70 τ. This signal was assigned to a proton on C-8, while another singlet far downfield at −2.93 τ was assigned to the hydroxyl proton on C-1. These assignments placed the remaining three methoxyl groups at C-5, C-6, and C-7.

NMR: −2.93 (s, OH), 3.64 (d, 2-H), 3.47 (d, 4-H), 2.70 (s, 8-H), 5.95 (s, 3,5,6,7-OCH_3), 6.04 (s), and 6.15 (s) ppm [lit. (3) NMR ($CDCl_3$): 3.76 (d, 2-H), 3.60 (d, 4-H), 2.70 (s, 8-H), 6.03 (s, 3,5,6,7-OCH_3), 6.13 (s), and 6.20 (s) ppm]; UV: λ_{max} (ethanol) 364 (log ϵ 4.03), 311 (4.44), 258 (4.74), and 239 (4.64) nm [lit. (3) UV: λ_{max} (methanol) 364 (log ϵ 3.81), 312 (4.18), 258 (4.49), and 240 (4.35) nm]; mp 178°; IR_{max}: 1653, 1608, and 1582 cm^{-1}.

1-Hydroxy-3,5,6,7,8-pentamethoxyxanthone (IV)—This compound exhibited an R_f value of 0.63 with benzene–ethyl acetate–acetic acid (90:5:5) as the solvent system and an R_f value of 0.73 with carbon tetrachloride–methanol (25:1). A melting point of 108–109° was observed. Mass spectrometry indicated the parent ion and base peak to be at *m/e* 362. This mass corresponds with a xanthone derivative having one hydroxyl and five methoxyl groups. It produced positive Wilson's boric acid and ferric chloride tests and was insoluble in aqueous sodium carbonate.

The IR spectrum exhibited a major band at 1653 cm^{-1}. NMR indicated four singlets at 6.05, 5.99, 5.93, and 5.80 τ, corresponding to five methyl group protons. Doublets at 3.58 and 3.43 τ were assigned to protons on C-2 and C-4, respectively. A single signal at −3.37 τ was indicative of a hydroxyl proton located on C-1. The characteristic pattern of doublets, the singlet far downfield, and data just mentioned indicated the presence of a hydroxyl group on C-1 and a methoxyl group on C-3. Thus, all positions on ring B were accounted for, leaving only ring A to accommodate the four remaining methoxyl groups. With only four positions available for substitution, no signal downfield would be anticipated if all four positions were occupied. This was indeed the case, as no downfield signal (other than that far downfield for the hydroxyl proton on C-1) was noted. Thus, the compound in question must be 1-hydroxy-3,5,6,7,8-pentamethoxyxanthone (IV). This compound has never been reported in the literature and has been named eustomin.

NMR: −3.37 (s, OH), 3.58 (d, 2-H), 3.43 (d, 4-H), 5.80 (s, 3,5,6,7,8-OCH_3), 5.93 (s), 5.99 (s), and 6.05 (s) ppm; UV: λ_{max} (ethanol) 350 sh (log ϵ 3.71), 312 (4.27), and 255 (4.55) nm; mp 108–109°; IR_{max}: 1653, 1595, and 1590 cm^{-1}.

1-Hydroxy-3,7,8-trimethoxyxanthone (V)—The fifth compound isolated exhibited an R_f value of 0.63 with benzene–ethyl acetate–acetic acid (90:5:5) as the solvent system and an R_f value of 0.65 with carbon tetrachloride–methanol (25:1). A melting point of 150–151° was noted. Mass spectrometry showed a parent ion and base peak at *m/e* 302. This mass corresponds to a xanthone compound having one hydroxyl and three methoxyl functions. Chemical and physical tests provided results identical to those mentioned for previous compounds, while the IR spectrum indicated a major band at 1642 cm^{-1}.

NMR analysis revealed two singlets at 6.15, 6.08, and 6.00 τ, representing three methyl group protons. A singlet at −3.10 τ represented a hydroxyl proton on C-1; a peak at 3.69 τ, which arose from two overlapping doublets, was assigned to protons on C-2 and C-4. Thus, once again a 1,3-dioxygenation pattern was evident with a hydroxyl group at C-1 and a methoxyl function at C-3. Therefore, only ring A was available to accommodate the two remaining methoxyl groups. Signals assigned to protons on C-5 and C-6 exhibited peaks at 2.83 and 2.74 τ, thus leaving only positions C-7 and C-8 to accommodate the methoxyl functions.

NMR: −3.10 (s, OH), 3.69 (d, 2-H), 3.69 (d, 4-H), 2.83 (s, 5-H), 2.74 (s, 6-H), 6.00 (s, 3,7,8-OCH_3), 6.08 (s), and 6.15 (s) ppm [lit. (4) NMR ($CDCl_3$): −3.30 (s, OH), 3.69 (d, 2-H), 3.69 (d, 4-H), 2.86 (s, 5H), 2.65 (s, 6-H), 5.99 (s, 3,7,8-OCH_3), 6.07, and 6.11 ppm]; UV: λ_{max} (ethanol) 373 (log ϵ 3.62), 312 (4.13), 261 (4.52), and 241 (4.44) nm [lit. (7) UV: λ_{max} (ethanol) 380 (log ϵ 3.7), 315 (4.1), 260 (4.6), and 242 (4.6) nm; (1) (ethanol) 313 (4.16), 362 (4.42), and 242 (4.29) nm]; mp 150–151° [lit. (5) mp 148–149°]; IR_{max}: 1642, 1603, and 1570 cm^{-1}.

Xanthone VI—This compound, collected in 1–2-mg yield, was not fully characterized. It exhibited an R_f value of 0.49. It did produce positive Wilson's boric acid and ferric chloride tests and was insoluble in aqueous sodium carbonate. The mass spectrometry was variable, as was an NMR analysis, undoubtedly due to impurities. UV scans indicated major bands at 237, 261, and 375 nm; its IR spectrum showed major bands at 1653, 1610, and 1580 cm^{-1}. Its NMR spectrum revealed the presence of two methyl group protons as singlets at 6.12 and 5.95 τ. A singlet far downfield at −3.78 τ represented a hydroxyl proton adjacent to a carbonyl function. This compound apparently contained the usual 1,3-dioxygenation pattern of ring B with a methyl group(s?) on ring A.

Yields of approximately 5, 10, 5, 6, 5, and 1 mg were obtained for Xanthones I, II, III, IV, V, and VI, respectively.

Glycosidic Studies—The solvent of the methanol extract of coarsely powdered root material was evaporated under vacuum. The resulting syrupy extract was solubilized in water and exhaustively partitioned with benzene. A final partitioning with chloroform left an extract in which no free xanthones (aglycones) could be detected when spotted on TLC and

[11] K and K Laboratories, Inc., Hollywood, Calif.

developed with either benzene–ethyl acetate–acetic acid or carbon tetrachloride–methanol. The developed plate was placed in an empty developing chamber. A 50-ml beaker of concentrated hydrochloric acid was placed in the chamber and then covered. The plate was thus exposed to concentrated hydrochloric acid fumes. Exposure was continued for 30 min.

After removal from the chamber, the plate was redeveloped in the solvent systems indicated. Six xanthone compounds, which corresponded on TLC with previously isolated material, were found. Also, by hydrolyzing the root extract from which all free xanthones had been removed with 3% aqueous hydrochloric acid and partitioning with benzene, the six xanthone (aglycone) derivatives could be detected. Thus, each of the six xanthones isolated from *E. grandiflorum* may exist in the plant as glycosides as well as free aglycones.

Phytochemical investigations of the various higher plants revealed the presence of xanthones in four primary families: Gentianaceae, Guttiferae, Moraceae, and Polygalaceae. The xanthones obtained from the Gentianaceae are limited to polyoxygenated derivatives, and the variation of substituents is restricted to hydroxyl and/or methoxyl groups (16). There are, however, exceptions to this general rule, as evidenced by the occurrence of mangiferin (a C-glucoside) in *Canscora decussata* Schult (1) and the presence of certain *O*-glucosides isolated from various members of this family (10, 17).

The oxygenation patterns of xanthones derived from higher plants indicate that they are formed by a mixed shikimate–acetate pathway (18). This approach suggests that ring A and the attached carbonyl group (C-7 unit) are provided *via* the shikimic acid pathway, whereas ring B (C-6 unit) originates through the acetate–malonate polyketide route. The C-7 and C-6 units thus generated form polyhydroxybenzophenones or benzophenone-like intermediates. The next step in biogenesis involves the intramolecular transformation of these intermediates into polyoxygenated xanthones (16, 18).

Various suggestions have been presented to account for the intramolecular transformation of the intermediates into xanthones. One method, direct phenolic oxidative coupling, is regarded as particularly valid. Carpenter *et al.* (18) emphasized that the observed oxygenation patterns of naturally occurring xanthones is precisely what would be expected for a phenoxyl radical coupling process in which the newly created carbon–oxygen bond would necessarily be located either *ortho* or *para* to oxygen functions in ring A. This could account for the frequently observed cooccurrence of pairs of corresponding oxygenated xanthones, *e.g.*, 5- and 7-monooxygenated xanthones, 1,5- and 1,7-dioxyxanthones, 1,5,6- and 1,6,7-trioxyxanthones, 1,3,5- and 1,3,7-trioxyxanthones, and 1,3,5,6- and 1,3,6,7-tetraoxyxanthones.

In support of the work just described, the xanthones isolated from *E. grandiflorum* were substituted with hydroxyl and methoxyl functions only. These substitutions closely parallel those of other members of the Gentianaceae. Also, the probable presence of glycosides was noted, and this observation coincided with previous reports concerning their presence in the Gentianaceae (10, 18).

This plant appears to follow the substitution pattern of some previously mentioned xanthones that contain corresponding oxygen pairs; that is, it contains 1,3,5- and 1,3,7-trioxygenated components as evidenced in II and I, respectively. Furthermore, it is interesting in that it produces a totally oxygenated and completely methoxylated A ring in IV.

No pharmacological or microbiological work was performed on these compounds, but other investigators noted such activity in total xanthone studies (19, 20). Compound II exhibited antitubercular activity, while V showed monoamine oxidase inhibiting activity devoid of pressor effects of tyramine and lacking in high toxicity. It would be interesting to note the actions of eustomin (IV) if subjected to similar studies due to its increased methoxylation. Increasing methoxylation may or may not enhance activity; however, it would perhaps be useful to determine if this activity is or is not related to the number of methoxyl groups in a xanthone compound.

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