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Coumarins XII: Synthesis of (±)-*cis*- and *trans*-3′,4′-Dihydroxy-3′,4′-dihydroxanthyletin and Their Diesters

SAMIR M. EL-ANTABLY* and T. O. SOINE▲

Abstract □ (±)-*cis*-3′,4′-Dihydroxy-3′,4′-dihydroxanthyletin was synthesized from xanthyletin by the use of osmium tetroxide. It was also obtained, unexpectedly, by acid or base hydrolysis of (±)-*trans*-3′-acetoxy-4′-(*m*-chlorobenzoyloxy)-3′,4′-dihydroxanthyletin and, spontaneously, by recrystallization of the product from epoxidation of xanthyletin with trifluoroperacetic acid. (±)-*trans*-3′,4′-Dihydroxy-3′,4′-dihydroxanthyletin was obtained together with the (±)-*trans*-3′-hydroxy-4′-acetoxy derivative by peracetic acid treatment of xanthyletin. The diacetates, dipropionates, and di-*n*-butyrates of both the *cis*- and *trans*-diols were prepared together with the *trans*-3′-propionoxy-4′-acetoxy- and 3′-*n*-butyroxy-4′-acetoxy-3′,4′-dihydroxanthyletins. NMR studies of the diols and the diesters were carried out to establish chemical shifts of specific groups, with special emphasis on the 2′-gem-dimethyls together with the *J* values for the 3′- and 4′-protons as diagnostic features for the *cis*- or *trans*-configuration.

Keyphrases □ Xanthyletin derivatives—synthesis and structure determination of (±)-*cis*- and *trans*-3′,4′-dihydroxy-3′,4′-dihydroxanthyletin and diesters □ Dihydroxy-3′,4′-dihydroxanthyletin, (±)-*cis* and *trans*, and diesters—synthesis and structure determination □ Coumarin derivatives—synthesis and structure determination of (±)-*cis*- and *trans*-3′,4′-dihydroxy-3′,4′-dihydroxanthyletin and diesters

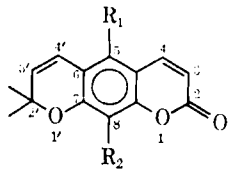
Naturally occurring pyranocoumarins, also known as chromeno-α-pyrones, have been known since the initial isolation of xanthoxyletin (I*b*) by Staples (1) in 1829, although its structure was not established until 1936 (2). Subsequent isolations and structural studies revealed three different basic ring fusions which may be said to be derived from xanthyletin (I*a*), alloxanthyletin (II*a*), and seselin (III*a*). The xanthyletin type is comprised of xanthyletin (I*a*), xanthoxyletin (I*b*), and luvangetin (I*c*). In the alloxanthyletin series, the known compounds are alloxanthoxyletin (II*b*), calophyllolide (II*c*), and inophyllolide (II*d*). Only two representatives of the seselin type are presently known: seselin (III*a*) and braylin (III*b*).

It was not until 1957 that the now well-known 3′,4′-diesters derived from 3′,4′-dihydroseselin were isolated and structurally characterized by Bencze *et al.* (3) when they described samidin (IV*a*) and visnadin (IV*b*). Their work was quickly followed by that of Smith *et al.* (4) who, in addition, characterized dihydrosamidin (IV*c*). Since then, a number of these diesters have been isolated and structurally elucidated (*e.g.*, 5–7) and their widespread presence in umbelliferous plants has been established. In addition, following the isolation and structural elucidation of lomatin (3′-hydroxy-3′,4′-dihydroseselin) (IV*d*) (8), esters of this coumarinic alcohol were reported (9, 10).

With respect to the corresponding esters related to the linear dihydro-I*a*, the first discoveries were the 3′-monoesters, namely, decursin (V*a*) (11) and the corresponding angelate (V*b*) (12). The present work was initiated in the hope that, eventually, the 3′,4′-diesters derived from 3′,4′-dihydroxy-3′,4′-dihydroxanthyletin would be isolated and that knowledge gained from the synthesis of known derivatives would be useful for characterization of such compounds. Indeed, during these studies the first such compound, xanthalin (V*c*), was reported from *Xanthogalum purpurascens* Lall. by Sokolova *et al.* (13) and provided an excellent corroboration of other findings (14). Since then Zheleva and coworkers (15–17) assigned similar structures to the coumarins of *Peucedanum arenarium*, and configurational assignments (17) have relied on the present work.

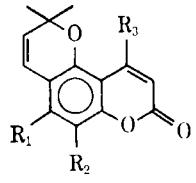
DISCUSSION

Xanthyletin (I*a*) was utilized as the starting point in the present work. This material is readily obtainable in a relatively pure form through the petroleum ether extraction procedure of King *et al.* (18) from the heartwood of East Indian satinwood, *Chloroxylon swietenia*. Recrystallization from warm methanol according to the procedure of Lemmich *et al.* (12) provided a means of separating xanthyletin from the accompanying xanthoxyletin in a form sufficiently pure for synthetic work. The xanthyletin thus obtained, when subjected to TLC on silica gel, showed only traces of xanthoxyletin when examined in the dark under UV light, both being highly fluorescent. The identity of xanthyletin was established by direct



I

Ia: $R_1 = R_2 = H$
Ib: $R_1 = OCH_3$, $R_2 = H$
Ic: $R_1 = H$, $R_2 = OCH_3$

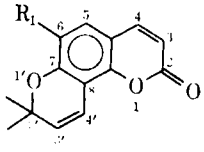


II

IIa: $R_1 = R_2 = R_3 = H$
IIb: $R_1 = OCH_3$, $R_2 = R_3 = H$

IIc: $R_1 = OCH_3$, $R_2 = C(=O)-C(CH_3)=CH-CH_3$, $R_3 = C_6H_5$

IId: $R_1 = OCH_3$, $R_2 = C(=O)-CH(CH_3)-CH_2CH_3$, $R_3 = C_6H_5$

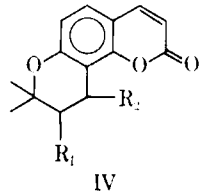


IIIa: $R_1 = H$
IIIb: $R_1 = OCH_3$

comparison with an authentic sample[1], and it was also identical in all respects to the same compound reported by Alpin and Page (19).

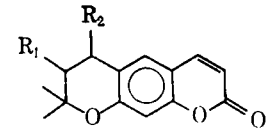
The objective was to utilize Ia to prepare both the *cis*- and *trans*-3′,4′-dihydroxy-3′,4′-dihydroxanthyletins (VIa and VIIa, respectively). It was anticipated that physical data on a number of diester derivatives could be obtained suitable for future structural studies since, aside from optical activity and melting-point differences, these would be useful models related to their natural counterparts.

Compound VIa was prepared by the action of osmium tetroxide on Ia. The diol so obtained showed a strong IR hydroxyl absorption band at 3450 cm.$^{-1}$, and the NMR spectrum (Table I) showed a coupling constant for the 3′- and 4′-protons of 4 Hz. as well as



IV

IVa: $R_1 = OOCCH=C(CH_3)_2$, $R_2 = OOCCH_3$
IVb: $R_1 = OOCCH(CH_3)CH_2CH_3$, $R_2 = OOCCH_3$
IVc: $R_1 = OOCCH_2CH(CH_3)_2$, $R_2 = OOCCH_3$
IVd: $R_1 = OH$, $R_2 = H$
IVe: $R_1 = R_2 = OOCCH_3$
IVf: $R_1 = R_2 = OH$



V

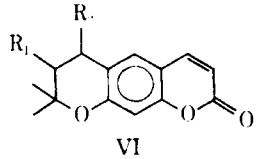
Va: $R_1 = OOC-CH=C(CH_3)_2$, $R_2 = H$

Vb: $R_1 = OOCC(CH_3)=CH-CH_3$, $R_2 = H$

Vc: $R_1 = R_2 = OOCC(CH_3)=CH-CH_3$

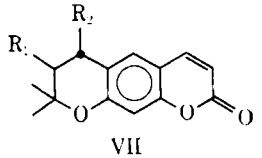
two singlets for the gem-dimethyl groups at 1.29 and 1.42 p.p.m. Compound VIa was converted to the diacetate (VIb), m.p. 172–173°, and it was also obtained in an attempt to prepare 3′,4′-epoxyxanthyletin by treating Ia with *m*-chloroperbenzoic acid. The oily product showed a hydroxyl absorption band at 3400 cm.$^{-1}$. The crude product was treated with sulfuric acid in dimethyl sulfoxide so that any epoxide present would be hydrolyzed to the diol. The product was a glass and showed an identical IR pattern to the untreated oily material, indicating that the epoxide ring opening had probably occurred in the original reaction mixture to generate a *trans*-hemiester of the diol in a manner similar to that previously reported (12). Acetylation of the crude product afforded *trans*-3′-acetoxy-4′-(*m*-chlorobenzoyloxy)-3′,4′-dihydroxanthyletin (VIIb). The IR spectrum did not show hydroxyl absorption, and the NMR spectrum (Table I) revealed a considerable difference in the chemical shifts of the gem-dimethyl protons and $J_{3',4'} = 6$ Hz.

Compound VIIb was subjected to both acid and base hydrolysis in an effort to obtain the *trans*-diol (VIIa), but only the *cis*-diol (VIa) was obtained. The products obtained in both cases showed identical IR and NMR spectra to those of VIa, and TLC failed to show detectable amounts of VIIa. Acetylation of these products gave diacetoxy derivatives, each identical to VIb as determined by melting point, mixed melting point, and IR and NMR spectra. Another surprising route to the *cis*-diol (VIa) was encountered during an attempted epoxidation of Ia with trifluoroperacetic acid (20). The product, m.p. 144–145°, when recrystallized from methanol, showed a large change in melting point which corresponded to that of the *cis*-diol (VIa). The identity of the product was confirmed by IR and NMR spectral properties and by acetylation to



VI

VIa: $R_1 = R_2 = OH$
VIb: $R_1 = R_2 = OOCCH_3$
VIc: $R_1 = R_2 = OOCCH_2CH_3$
VId: $R_1 = R_2 = OOCCH_2CH_2CH_3$



VII

VIIa: $R_1 = R_2 = OH$
VIIb: $R_1 = OOCCH_3$, $R_2 = OOC$-*m*-C_6H_4Cl
VIIc: $R_1 = OH$, $R_2 = OCH_3$
VIId: $R_1 = OOCCH_3$, $R_2 = OCH_3$
VIIe: $R_1 = OH$, $R_2 = OOCCH_3$
VIIf: $R_1 = R_2 = OOCCH_3$
VIIg: $R_1 = R_2 = OOCCH_2CH_3$
VIIh: $R_1 = R_2 = OOCCH_2CH_2CH_3$
VIIi: $R_1 = OOCCH_3$, $R_2 = OOCCH_2CH_3$
VIIj: $R_1 = OOCCH_3$, $R_2 = OOCCH_2CH_2CH_3$

[1] Supplied through the courtesy of Dr. B. E. Nielsen, Royal Danish School of Pharmacy, Copenhagen, Denmark.

Table I—Chemical Shifts (δ) in $CDCl_3$ for Xanthyletin and Its Derivatives

Compound	3-H (d)	4-H (d)	5-H (s)	8-H (s)	3′-H (d)	4′-H (d)	$J_{3,4}$ (Hz.)	$J_{3',4'}$ (Hz.)	2′-$(CH_3)_2$
I*a*	5.65	7.55	7.00	6.65	6.17	6.30	9.5	10	1.45
VI*a*[a]	6.26	7.95	7.71	6.69	3.72	4.84	9.5	4	1.29, 1.42
VI*b*	6.25	7.60	7.30	6.79	5.32	6.19	9.5	4	1.45
VI*c*	6.25	7.60	7.30	6.80	5.39	6.20	9.5	4	1.45
VI*d*	6.25	7.60	7.30	6.80	5.39	6.20	9.5	4	1.45
VII*a*[a]	6.25	7.95	7.71	6.70	3.52	4.84	9.5	9	1.22, 1.47
VII*b*	6.25	7.60	6.85	6.30	5.40	6.25	9.5	6	1.42, 1.52
VII*c*[a]	6.10	7.85	7.50	6.55	3.76	4.28	9.5	7	1.10, 1.25
VII*d*	6.25	7.62	7.55	6.80	4.35	5.25	9.5	5	1.38, 1.45
VII*e*	6.25	7.60	7.20	5.92	3.90	5.95	9.5	7	1.35, 1.51
VII*f*	6.25	7.60	7.35	6.80	5.25	6.02	9.5	6	1.38, 1.45
VII*g*	6.25	7.60	7.35	6.80	5.25	6.02	9.5	6	1.38, 1.45
VII*h*	6.25	7.60	7.35	6.80	5.25	6.02	9.5	6	1.38, 1.45
VII*i*	6.25	7.60	7.35	6.80	5.25	6.02	9.5	6	1.38, 1.45
VII*j*	6.25	7.60	7.35	6.80	5.25	6.02	9.5	6	1.38, 1.45

[a] The solvent in these cases was dimethyl sulfoxide-d_6.

VI*b*. *cis*-3′,4′-Diacetoxy-3′,4′-dihydroseselin (IV*e*) was prepared to compare its NMR spectrum with that of VI*b*. Compound IV*e* did not differ greatly from VI*b* (Table I) in that it showed (in $CDCl_3$) 1.40 [6H, 2s, $C_{2'}$—$(CH_3)_2$], 2.04 and 2.09 (6H, 2s, 2-$OCOCH_3$), 5.20 (1H, d, $C_{3'}$—H), and 6.38 (1H, d, $C_{4'}$—H) p.p.m. with $J_{3',4'}$ = 5 Hz.

The reason for the virtually exclusive formation of the *cis*-diol (VI*a*) over the *trans*-diol (VII*a*) in these hydrolytic procedures is not readily apparent, since mixtures of the *cis*- and *trans*-diols occur routinely from alkaline saponification of 3′,4′-diacyloxy-3′,4′-dihydroseselins (3). Although speculative, this might be ascribed to the hydrogen-bonding stability in both the *cis*- and *trans*-diols derived from the seselin series in which the 4′-hydroxy group can hydrogen bond with the lactone ether oxygen, a situation that cannot possibly take place in the series derived from xanthyletin. The behavior of VII*b* on treatment with methanolic potassium hydroxide may have bearing on the possibility of hydrogen bonding of the hydroxyl groups being influential in the virtually exclusive formation of the *cis*-diol (VI*a*). Because a methoxyl group would now occupy the 4′-position, the possibility of hydrogen bonding would be minimized. Ordinarily, as in the seselin-derived series, based on the suggested enone mechanism (4), a mixture of *cis*- and *trans*-isomers should result. In the present case, however, the IR and NMR spectra (Table I) were completely compatible with the assignment of a *trans*-structure. This compound (VII*c*) was acetylated to provide *trans*-3′-acetoxy-4′-methoxy-3′,4′-dihydroxanthyletin (VII*d*) which, by IR, showed no hydroxyl absorption; the NMR showed (in dimethyl sulfoxide-d_6) the presence of the methyl ether singlet at 3.58 p.p.m. in VII*c* and at 3.52 p.p.m. in VII*d* as well as a singlet for acetoxymethyl at 2.12 p.p.m. A *trans*-assignment for the 3′,4′-substituents was tentatively made on the basis of the substantial separation of the gem-dimethyl singlets and the variable $J_{3',4'}$ for VII*c* and VII*d*.

The *cis*-dipropionoxy and *cis*-di-*n*-butyroxy esters (VI*c* and VI*d*) were prepared in addition to VI*b*. The IR spectra of the prepared esters showed no hydroxyl absorption, and they all possessed virtually the same NMR pattern (Table I) except for differences in the acid moieties. The differences in chemical shifts of the gem-dimethyl singlets (1.29 and 1.42 p.p.m.) noted in the diol was eliminated in the diesters (1.45 p.p.m.). Previously (12), *cis*-3′,4′-dihydroxy-3′,4′-dihydroseselin (*i.e.*, *cis*-khellactone, IV*f*) showed only a small difference in the chemical shifts of the gem-dimethyl groups (1.41 and 1.44 p.p.m.) with $J_{3',4'}$ = 5 Hz. This same diol was prepared in the present study. No difference was found in the chemical shifts of the gem-dimethyl groups with only a singlet at 1.42 p.p.m. It was also reported (12) that *trans*-3′,4′-dihydroxy-3′,4′-dihydroseselin (*i.e.*, *trans*-khellactone) showed 1.31 and 1.51 p.p.m. for the gem-dimethyl groups and $J_{3',4'}$ = 6.9 Hz. In the present study, *trans*-khellactone (3) and its diacetate were prepared, and the NMR spectra were found to be identical to those reported (12).

The preparation of the *trans*-diol (VII*a*) was finally achieved while attempting to prepare 3′,4′-epoxyxanthyletin using 40% peracetic acid in acetic acid. Epoxidation of xanthyletin with peracetic acid resulted, as previously observed with *m*-chloroperbenzoic acid (12), in spontaneous opening of the epoxide by acetate anion. Workup of the reaction mixture first provided *trans*-3′-hydroxy-4′-acetoxy-3′,4′-dihydroxanthyletin (VII*e*), the IR of which showed hydroxyl absorption at 3400 $cm.^{-1}$; the NMR spectrum (Table I) possessed two widely separated signals for the gem-dimethyls (*i.e.*, 1.35 and 1.51 p.p.m.) and $J_{3',4'}$ = 7 Hz. The mother liquor, upon concentration, yielded a crystalline diol which had a different melting point than VI*a* and depressed its melting point on admixture. The IR of the diol exhibited a strong hydroxyl band at 3400 $cm.^{-1}$, and the NMR spectrum (Table I) showed two singlets for the gem-dimethyls at 1.22 and 1.47 p.p.m. and $J_{3',4'}$ = 9 Hz. Acetylation of the diol and VII*e* resulted in the same diacetoxy derivative (VII*f*) as evidenced from the melting point, mixed melting point, and IR and NMR spectra. VII*f* possessed different physical constants and spectra from those of the *cis*-diacetoxy derivative (VI*a*). Additional evidence for the *trans*-structure was obtained from the preparation of *trans*-dipropionoxy-, *trans*-di-*n*-butyroxy-, *trans*-3′-acetoxy-4′-propionoxy-, and *trans*-3′-acetoxy-4′-*n*-butyroxy-3′,4′-dihydroxanthyletins (VII*g*, VII*h*, VII*i*, and VII*j*, respectively). Each *trans*-diester (VII*g* and VII*h*) possessed a different melting point from that of the corresponding *cis*-diester (*i.e.*, VI*c* and VI*d*). All of the *trans*-diesters prepared had nearly identical NMR spectra except for differences due to the acyloxy groups, and they showed $J_{3',4'}$ = 6 Hz. and widely separated signals for the gem-dimethyl groups at 1.38 and 1.45 p.p.m. They were significantly different from the *cis*-diesters, which showed $J_{3',4'}$ = 4 Hz. and only one signal for the gem-dimethyl groups at 1.45 p.p.m.

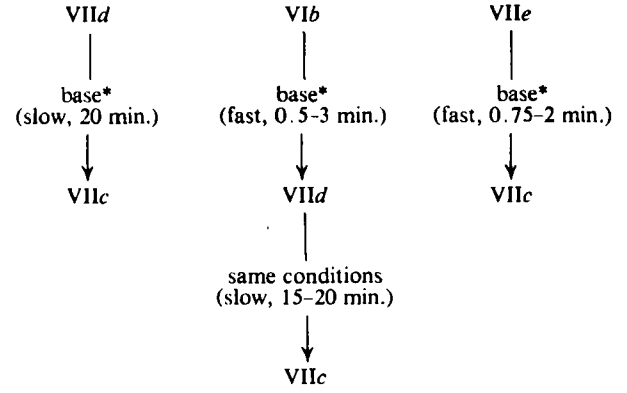
It was hoped that mild acid hydrolysis of VII*e* would result in the *trans*-diol (VII*a*) but, unfortunately, the NMR spectrum of the diol obtained showed that the hydrolysis resulted in a mixture of *cis*- and *trans*-diols; characteristic signals for each were observed in the spectrum of the product. Presumably, the milder conditions in this hydrolysis did not result in complete conversion of VII*e* to the *cis*-diol as was the case in the acid hydrolysis of VII*b* where VI*a* appeared to be virtually the exclusive product. On the basis of these experimental findings, together with the NMR data, it appears very probable that the product isolated from the mother liquor of the peracetic acid epoxidation reaction was the *trans*-diol (VII*a*). Therefore, the identity and physical characteristics of the *cis*- and *trans*-diols (VI*a* and VII*a*, respectively) are established.

As a result ot this study, it has been possible to draw conclusions regarding the NMR spectra of the 3′,4′-diols and diesters derived from 3′,4′-dihydroxanthyletin, as well as to establish the relationships between these compounds and those of the isomeric series derived from seselin.

With the *cis*-diol and its diesters, $J_{3',4'}$ was invariably 4 Hz. regardless of the substituent, a situation quite similar to that obtained with naturally occurring 3′,4′-dihydroseselin diesters where $J_{3',4'}$ was invariably 5 Hz. The *trans*-diol and its diesters prepared in this study showed a variability of $J_{3',4'}$ from 6 to 9 Hz., together with well-separated singlets for the gem-dimethyl group ranging from 1.22 to 1.51 p.p.m. If only xanthyletin *trans*-diesters are considered, however, $J_{3',4'}$ was consistently 6 Hz., combined with a well-defined spread ranging from 1.38 to 1.45 p.p.m. for the gem-dimethyl singlets. The generalization of Lemmich *et al.* (12) seems to hold with respect to the diagnostic requirement that well-separated gem-

dimethyl signals are indicative of the *trans*-arrangement and that little or no separation of these signals suggests the *cis*-arrangement. However, within the limited spectra of the diesters prepared in this study, the variability of $J_{3',4'}$ noted in the seselin-derived *trans*-compounds (12) has not been observed, with the single exception of the parent diol (VII*a*) itself[2].

Although there seemed to be little question concerning the assignments of the respective locations (*i.e.*, 3′ and 4′) of the acetoxy moieties in VII*d* and VII*e*, it was desirable to substantiate them independently. A relatively simple experiment was carried out by taking advantage of the greatly differing rates of hydrolysis of 3′- and 4′-acyloxy substituents in these compounds. The 3′-ester groups are subject to a normal basic hydrolysis whereas the 4′-ester groups are much more labile on the basis of the special mechanism referred to earlier (4). Therefore, by carrying out the reaction of methanolic potassium hydroxide on VII*d*, VII*e*, and VI*b*, it appeared possible to follow the rates of change by means of TLC and, thereby, to show that in VII*d* the rate of saponification is relatively slow whereas in VII*e* the rate of generation of the deacylated compound is comparatively much more rapid. The experience with VI*b* was in complete accord with the expected fast loss of 4′-acetate and the slower loss of the remaining 3′-acetate group. The data recorded in Scheme I summarize the results, which clearly substantiate the positions assigned to the acetoxy moieties in VII*d* and VII*e*.



*Scheme I—Base (*0.01 N methanolic potassium hydroxide) hydrolysis of VII*d, *VII*e, *and VI*b *at room temperature*

EXPERIMENTAL[3]

Extraction of Xanthyletin (I*a*)—Fine wood shavings (600 g.) of *Chloroxylon swietenia* were exhaustively extracted[4] for 5 days with petroleum ether (b.p. 30–60°) in a soxhlet apparatus. The solvent was removed by distillation, and the yellow crystalline material remaining was recrystallized from warm methanol to obtain colorless needles of xanthyletin, m.p. 128–130° [lit. (18) m.p. 126–127°]. TLC, in comparison with an authentic specimen[1], indicated the identity of the two samples. TLC was carried out on silica gel impregnated with formamide and developed with either dibutyl ether or *n*-hexane[5]-cyclohexane (1:1); a mixed melting-point comparison was also made.

***cis*-3′,4′-Dihydroxy-3′,4′-dihydroxanthyletin (VI*a*)**—A solution of I*a* (1.45 g., 0.0063 mole) in dioxane (50 ml.) was added to a solution of osmium tetroxide (2 g., 0.0079 mole) in dioxane (50 ml.), and the mixture was kept in the dark at room temperature for 4 days. Ethyl acetate (50 ml.) was added, and a stream of hydrogen sulfide was bubbled into the reaction mixture for 2 hr. The black precipitate was removed by filtration, and the filtrate was evaporated to dryness to obtain a white crystalline material (1 g., 60.5%) which was recrystallized several times from methanol to obtain a product, m.p. 234–236°, shown to be VI*a*.

Anal.—Calc. for $C_{14}H_{14}O_5$: C, 64.11; H, 5.38. Found: C, 64.09; H, 5.52.

***cis*-3′,4′-Diacetoxy-3′,4′-dihydroxanthyletin (VI*b*)**—A mixture of VI*a* (0.2 g., 0.0008 mole), acetic anhydride (4 ml.), and anhydrous sodium acetate (0.25 g.) was refluxed for 2 hr. The hot solution was poured into ice-cold water, filtered, washed with water, and recrystallized from methanol to give 0.22 g. (85%) of VI*b*, m.p. 172–173°.

Anal.—Calc. for $C_{18}H_{18}O_7$: C, 62.42; H, 5.24. Found: C, 62.46; H, 5.45.

***trans*-3′-Acetoxy-4′-(*m*-chlorobenzoyloxy)-3′,4′-dihydroxanthyletin (VII*b*)**—To an ice-cold solution of xanthyletin (0.6 g., 0.0026 mole) in chloroform (50 ml.) was added dropwise an ice-cold solution of *m*-chloroperbenzoic acid (80%, 0.56 g.) in chloroform (75 ml.), and the mixture was stirred at room temperature in the dark for 24 hr. The reaction mixture was successively washed with 100 ml. each of 10% sodium bicarbonate, 5% sodium iodide, 5% sodium sulfite, and, finally, water. The dried (sodium sulfate) chloroformic solution was evaporated to dryness, and the residue was dissolved in dimethyl sulfoxide (75%, 40 ml.) containing sulfuric acid (1 ml.). The mixture was stirred at room temperature for 16 hr. and then diluted with water until precipitation ceased. The white solid which separated was filtered, washed with water, and dried. The crude product was refluxed for 2 hr. with acetic anhydride (15 ml.) and anhydrous sodium acetate (1 g.), the mixture was poured into ice-cold water and filtered, and the white crystalline material obtained was recrystallized from methanol to yield 0.6 g. (54.5%) of white prisms, m.p. 165–167°.

Anal.—Calc. for $C_{23}H_{19}ClO_7$: C, 62.38; H, 4.32. Found: C, 62.34; H, 4.04.

Acid Hydrolysis of VII*b*—A solution of VII*b* (0.45 g., 0.001 mole) in a mixture of ethanol (8 ml.) and sulfuric acid (0.8 ml.) was refluxed for 3 hr. The cooled reaction mixture was diluted with water (38 ml.), and the alcohol was removed under reduced pressure. After standing for 30 min. at room temperature, the crystalline precipitate was filtered and recrystallized from methanol to obtain 0.20 g. (75%) of colorless prisms, m.p. 188–191°. In spite of the broad range of the melting point, the IR and NMR spectra were largely identical to those of VI*a*. The product was then acetylated in the same manner as for the preparation of VI*b* to yield a diacetate identical to VI*b* in melting point, mixed melting point, TLC behavior, and IR and NMR spectra. These data suggest that VI*a* obtained in this way is a dimorphic form of the higher melting product.

Base Hydrolysis of VII*b*—A mixture of VII*b* (0.6 g., 0.0014 mole) in dioxane (5 ml.) and 1 *N* aqueous potassium hydroxide (15 ml.) was allowed to stand overnight at room temperature. The mixture was then diluted with water (1000 ml.), acidified with 20% sulfuric acid, and allowed to stand for 1 hr. at room temperature. The reaction mixture was brought to pH 8 with aqueous sodium carbonate and then extracted with chloroform. The chloroformic extract was washed with water, dried (sodium sulfate), and evaporated to dryness to obtain a crystalline residue, which was crystallized from methanol to yield 0.3 g. (84.3%) of colorless crystals, m.p. 234–

[2] Lemmich *et al.* (12) discussed the relative $J_{3',4'}$ values of the *cis*- and *trans*-3′,4′-disubstituted-3′,4′-dihydroseselins in connection with their similarity to 3,4-flavandiols and their derivatives. The latter are almost frozen conformers with the 2-aryl substituent equatorial. In the above seselin derivatives and, presumably, with the corresponding xanthyletin derivatives discussed here, the observed *J* values correspond to time averages of the two possible conformations. They note that the dihedral angles of the two *trans*-conformers vary considerably whereas those of the *cis*-form are virtually the same; therefore, the conformational equilibria determine the resultant *J* values, with those for the *cis*-derivatives being relatively constant and those for the *trans*-derivatives being potentially variable depending on the substituents.

[3] Melting points were determined in capillary tubes in a Thomas-Hoover melting-point apparatus and are uncorrected. IR (KBr pellet) and UV (95% ethanol) spectra were determined on a Perkin-Elmer 237B grating IR spectrophotomer and a Cary model 14, respectively. The observed absorption bands, in each case, were in accord with the assigned structures and only values with a diagnostic function were mentioned in the previous discussion. NMR spectra were obtained on a Varian A-60D instrument using tetramethylsilane as an internal standard; the observed values, together with the solvents used, are recorded in Table I. TLC was performed on Eastman silica gel Chromagram sheets (No. 6060) unless otherwise specified as with the plates employing silica gel impregnated with formamide. In this case, a mixture of silica gel G (E. Merck AG) (30 g.), formamide (30 ml.), and distilled water (60 ml.) was shaken for 45 sec., spread over 20 glass plates 5.08 × 20.32 cm. (2 × 8 in.), and left to dry at room temperature overnight. Preparative TLC plates refer to alumina or silica gel plates (F_{254}, Brinkmann, 20 × 20 cm., 2000 μ thick). The *Chloroxylon swietenia* (Rutaceae) wood was obtained from Craftsman Wood Service Co., Chicago, Ill., and was authenticated by Mr. A. Stamm, Forest Products Laboratory, Madison, Wis. It was reduced to fine shavings with a mechanical planer prior to extraction. Other reagent chemicals were obtained from reputable sources. Analyses were performed by the Microanalytical Laboratory, University of Minnesota, and Schwartzkopf Microanalytical Laboratory, Woodside, N. Y.

[4] Skellysolve F.

[5] Skellysolve B.

236°. The product was identical to VI*a*, and acetylation in the usual manner afforded a diacetate identical to VI*b*.

***trans*-3′-Hydroxy-4′-methoxy-3′,4′-dihydroxanthyletin (VII*c*)**—A mixture of VII*b* (3g., 0.0067 mole) in methanol (120 ml.) and 1 *N* methanolic sodium hydroxide (12 ml.) was refluxed for 45 min. The cooled reaction mixture was diluted with water (60 ml.), and the alcohol was removed under reduced pressure. The aqueous layer was further diluted with water (1000 ml.), acidified with 20% sulfuric acid, and allowed to stand for 30 min. at room temperature. The pH of the mixture was adjusted to 8 with sodium carbonate solution, and the mixture was extracted thoroughly with chloroform. The extract was washed with water, dried (sodium sulfate), and stripped of solvent to yield 1.08 g. (57.7%) of a yellow crystalline material, m.p. 180–182°. The crude product was purified on 12 alumina plates which were developed in a benzene–ether (4:1) mixture. The blue fluorescent band was removed and the material was extracted with a warm methanol–acetone (1:1) mixture. The solvents were removed by evaporation, and the residue was recrystallized several times from methanol to yield 0.84 g. (44.9%) of white prisms, m.p. 185–186°.

Anal.—Calc. for $C_{15}H_{16}O_5$: C, 65.21; H, 5.48. Found: C, 65.20; H, 5.63.

The preceding compound (VII*c*) (0.1 g., 0.00036 mole) was refluxed for 2 hr. with acetic anhydride (5 ml.) and anhydrous sodium acetate (0.25 g.). The hot solution was poured into ice-cold water, and the precipitated crystalline material was filtered and washed with water. Recrystallization from methanol–water provided 0.09 g. (80%) of a colorless acetate, m.p. 134–135°.

Anal.—Calc. for $C_{17}H_{18}O_6$: C, 64.14; H, 5.69. Found: C, 64.34; H, 5.70.

***trans*-3′-Hydroxy-4′-acetoxy-3′,4′-dihydroxanthyletin (VII*e*) and *trans*-3′,4′-Dihydroxy-3′,4′-dihydroxanthyletin (VII*a*)**—A mixture of I*a* (2.85 g., 0.0125 mole), sodium acetate trihydrate (2.5 g.), and chloroform (50 ml.) was stirred in an ice bath. Peracetic acid in acetic acid (40%, 7.5 ml., ~ 0.04 mole) was added dropwise, and the reaction mixture was stirred in an ice bath for 2 hr. and then at room temperature for a total of 14 hr. The mixture was successively washed with water, 5% sodium bicarbonate, and water. The extract was dried (sodium sulfate) and stripped of solvent to obtain a colorless glass, which crystallized on treatment with benzene–hexane[5] (1:1) and was recrystallized from methanol to give VII*e* (1.8 g., 47.3%), m.p. 166–168°, as colorless prisms.

Anal.—Calc. for $C_{16}H_{16}O_6$: C, 63.15; H, 5.30. Found: C, 63.13; H, 5.38.

The combined mother liquors were evaporated to dryness, and the crude residue was purified by chromatography on six preparative silica gel plates. The plates were developed with a chloroform–methanol mixture (39:1) and air dried, and the two blue fluorescent bands which formed were removed from the plates. Each band was eluted with warm methanol–acetone (1:1) and, when the solvents were evaporated, the component with the higher R_f value (0.2 g.) was found to be identical to VII*e* (total yield 2 g., 52.6%). The second component was crystallized several times from methanol–water to obtain colorless crystals (0.98 g., 30%) of VII*a*, m.p. 194–196°.

Anal.—Calc. for $C_{14}H_{14}O_5$: C, 64.11; H, 5.38. Found: C, 63.97; H, 5.60.

Acetylation of VII*a* and VII*e* in the usual manner afforded, in each case, a virtually quantitative yield of *trans*-3′,4′-diacetoxy-3′,4′-dihydroxanthyletin (VII*f*) as colorless crystals from methanol, m.p. 189–191°.

Anal.—Calc. for $C_{18}H_{18}O_7$: C, 62.42; H, 5.24. Found: C, 62.21; H, 5.32.

Acid Hydrolysis of VII*e*—A mixture of VII*e* (0.18 g., 0.00058 mole) in 5% hydrochloric acid (3 ml.) was stirred on a steam bath for 45 min. and poured into water (50 ml.); the resulting mixture was extracted with ether (4 × 100 ml.). The ethereal extract was washed once with 5% sodium bicarbonate (100 ml.) and then water, dried (sodium sulfate), and stripped of solvent. The crystalline residue was recrystallized from dilute methanol to yield colorless crystals (0.11 g., 73.3%), m.p. 181–183°. Its NMR spectrum indicated that it was a mixture of VI*a* and VII*a* and, because of the small amount of material, separation into its components was not attempted.

Epoxidation of Xanthyletin with Trifluoroperacetic Acid—An ice-cold stirred suspension of hydrogen peroxide (90%, 0.6 ml., 0.02 mole) in methylene chloride (5 ml.) was treated with trifluoroacetic anhydride (3.7 ml., 0.026 mole); the mixture was stirred for 10 min. and then transferred to a dropping funnel. The mixture was added dropwise (20 min.) to a stirred ice-cold solution of I*a* (4.56 g., 0.02 mole) and triethylammonium trifluoroacetate (2.14 g., 0.01 mole) in methylene chloride (25 ml.), and the mixture was stirred at room temperature for 17.5 hr. according to the method of Emmons *et al.* (20). The volatile solvents were removed under reduced pressure, and the brown residual oil was dissolved in benzene (200 ml.) and then washed with water (2 × 75 ml.), dried (sodium sulfate), and stripped of solvent. The brownish residue was crystallized from benzene–*n*-hexane[5] to give 3.39 g. (70%) of pale-yellow crystals, m.p. 144–145°[6]. Recrystallization from warm methanol, however, afforded colorless crystals, m.p. 226–228°. These, when acetylated in the usual fashion with anhydrous sodium acetate and acetic anhydride, gave a crystalline diacetate, m.p. 172–173°, identical to VI*b*.

***cis*-3′,4′-Dipropionoxy-3′,4′-dihydroxanthyletin (VI*c*)**—A mixture of VI*a* (0.1 g., 0.0004 mole), propionic anhydride (1.5 ml., 0.012 mole), and pyridine (0.5 ml.) was refluxed for 4 hr. and poured into an excess of ice-cold water. The brown oil which separated was extracted with ether (4 × 100 ml.), and the extract was then successively washed with 100-ml. portions of 5% hydrochloric acid, 5% sodium bicarbonate, and water. The extract was dried (sodium sulfate) and filtered, and the ether was evaporated to obtain a brown semisolid residue (1.25 g.). This residue was chromatographed on 10 preparative silica gel plates with a mixture of benzene–ethyl acetate (7:3). The blue fluorescent band (visualized under UV light) was removed and extracted with a warm mixture of acetone–methanol (1:1). The solvents were evaporated, and the residue was recrystallized several times from methanol–water to give colorless crystals of VI*c* (0.9 g., 60%), m.p. 115–116°.

Anal.—Calc. for $C_{20}H_{22}O_7$: C, 64.16; H, 5.92. Found: C, 63.87; H, 5.93.

***cis*-3′,4′-Di-*n*-butyroxy-3′,4′-dihydroxanthyletin (VI*d*)**—This was prepared in a manner similar to that for VI*c* from VI*a* using *n*-butyric anhydride and pyridine. The product, m.p. 80–81°, was obtained in 80% yield.

Anal.—Calc. for $C_{22}H_{26}O_7$: C, 65.65; H, 6.51. Found: C, 65.59; H, 6.32.

***trans*-3′-Propionoxy-4′-acetoxy-3′,4′-dihydroxanthyletin (VII*i*)**—This was prepared as for VI*c* from VII*e*, using propionic anhydride and pyridine, in 76% yield, m.p. 108–109°.

Anal.—Calc. for $C_{19}H_{20}O_7$: C, 63.33; H, 5.59. Found: C, 63.44; H, 5.61.

***trans*-3′-*n*-Butyroxy-4′-acetoxy-3′,4′-dihydroxanthyletin (VII*j*)**—This was prepared as for VI*c* from VII*e*, using *n*-butyric anhydride and pyridine, in 80% yield, m.p. 131–132°.

Anal.—Calc. for $C_{20}H_{22}O_7$: C, 64.14; H, 5.92. Found: C, 64.41; H, 5.94.

***trans*-3′,4′-Dipropionoxy- and *trans*-3′,4′-Di-*n*-butyroxy-3′,4′-dihydroxanthyletins (VII*g* and VII*h*)**—These compounds were prepared from VII*a* and the corresponding anhydride as described previously for VI*c* to obtain crystalline derivatives in 62% yield, m.p. 122–124°, and 69% yield, m.p. 76–77°, respectively.

Anal.—Calc. for VII*g*, $C_{20}H_{22}O_7$: C, 64.14; H, 5.92. Found: C, 64.45; H, 6.17.

Anal.—Calc. for VII*h*, $C_{22}H_{26}O_7$: C, 65.65; H, 6.51. Found: C, 65.59; H, 6.73.

Action of Methanolic Potassium Hydroxide on VII*d*, VI*b*, and VII*e*—To a solution of VII*d* (0.1 g.) in methanol (4 ml.) was added dropwise at room temperature a solution of 0.1 *N* methanolic potassium hydroxide until the mixture became alkaline to litmus paper. Portions of the solution were withdrawn at intervals, acidified with 2% hydrochloric acid, spotted on silica gel plates, developed with benzene–methanol (9:1), and examined in the dark under UV light. The starting material remained unaffected for the first 20 min. and then slowly disappeared as two spots with lower mobility appeared corresponding to VII*c* and VI*a*. The same experiment was repeated

[6] The identity of this product, unfortunately, was not determined unambiguously before it was recrystallized from methanol, during which process it increased markedly in melting point. Although speculative, it is possible that it could have been *trans*-3′-hydroxy-4′-trifluoroacetoxy-3′,4′-dihydroxanthyletin, obtained in much the same way as the 3′-hydroxy precursor to VII*b* described elsewhere in this paper. Hydrolysis during the heating with methanol could conceivably have then produced the higher melting VI*a*.

using VI*b*. TLC analysis after 30 sec. indicated only a very faint fluorescent spot of VI*b* remaining and a strongly fluorescent spot corresponding to VII*d*. The starting material disappeared completely in 3 min. After 15 min., two additional spots appeared corresponding to VII*c* and VI*a*. Finally, VII*e* was treated in the same manner as VII*d* and VI*b*. TLC analysis after 45 sec. revealed two spots, a faint one corresponding to VII*e* and a stronger spot corresponding to VII*c*. After 2 min., the starting material had disappeared and an additional spot corresponding to VI*a* appeared.

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Altered Bioavailability of Drugs in the Eye Due to Drug-Protein Interaction

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Abstract □ The biological activity of a number of ophthalmic drugs is influenced by drug-protein interaction in tissues and fluids of the eye. High concentration of protein in lacrimal fluid, in both normal and pathological states, coupled with a relatively rapid turnover of this fluid, which moves drug solution away from the eye, leads to a considerable loss in drug activity for drugs that bind to protein. High levels of protein, as occur in some pathological states, and a slower, but substantial, turnover rate of aqueous humor can also lead to significant drug loss and a decrease in drug activity for compounds that complex with proteins. The present study, utilizing both *in vitro* and *in vivo* experiments, shows that drug-protein interaction in tissues and fluids of the eye occurs and that this interaction has an enormous influence on drug bioavailability. Equilibrium dialysis experiments, using pilocarpine nitrate, sulfisoxazole, and methylprednisolone, demonstrated that extensive binding to proteins in tears, cornea, and aqueous humor does occur. In addition, pupillary diameter experiments, using pilocarpine nitrate as the test agent, illustrated the influence of drug-protein interaction on drug bioavailability. A discussion of drug binding in combination with tear and instilled fluid dynamics is presented. It is suggested that this phenomenon can be responsible, partly or wholly, for some reported anomalous observations associated with drug therapy in the eye.

Keyphrases □ Ophthalmic bioavailability—effects of drug-protein binding, equilibrium dialysis, pupillary diameter experiments in rabbits □ Drug-protein binding—effect on bioavailability of ophthalmic drugs, equilibrium dialysis and pupillary diameter experiments

Binding of drugs to proteins can greatly affect drug activity. When this interaction occurs in tissues and fluids that are turned over at appreciable rates, and from which drug absorption must occur, the problem becomes even more severe, since both free drug and its reservoir of bound drug are being lost. Lacrimal fluid in both normal and pathological states contains appreciable quantities of protein and is turned over at rapid rates, while aqueous humor in some pathological conditions also contains large amounts of protein and is turned over at a slower but still significant rate. One could expect then that drug protein interaction in eye tissues and fluids would reduce the amount of free drug to either act locally or reach the anterior chamber