Anthraquinone Drugs. II: Inadvertent Acetylation of Aloe-Emodin During Preparation of Aglycones from Crude Drugs—UV, IR, and NMR Spectra of the Products

G-8

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Abstract □ Hydrolysis of anthraquinone glycosides with acetic acid (Auterhoff's method) leads to a partial acetylation of aloe-emodin, one of the liberated aglycones. With the aid of UV, IR, and NMR spectra this hitherto unreported compound has been characterized as aloe-emodin monoacetate. The same compound is also formed when the simultaneous oxidation and hydrolysis of the glycosides is effected with $FeCl_3$ in presence of HCl and the resultant aglycones extracted with ethyl acetate.

Keyphrases □ Anthraquinone glycosides—extraction □ Acetic acid extraction—aloe-emodin acetylation □ Aloe-emodin monoacetate—inadvertent formation □ TLC—separation □ UV spectrophotometry—identity, structure □ IR spectrophotometry—identity, structure □ NMR spectroscopy—identity, structure □ Mass spectroscopy—molecular weight

An important step in the analysis and structure elucidation of anthraquinone glycosides in vegetable laxatives is the conversion of these compounds into corresponding aglycones. For the hydrolysis of the glycosides and simultaneous extraction of the resulting aglycones, acetic acid has been used (Auterhoff's method) (1). In relatively older procedures hydrolysis of the glycosides is achieved with a mineral acid and the resultant aglycones are repeatedly extracted with lipophilic solvents (1). Glycosides containing a sugar moiety linked to the aglycone through a C—C bond require simultaneous oxidation and hydrolysis. Usually acidic ferric chloride solution is employed for the purpose (2, 3).

In a study of cascara sagrada in this laboratory, it was observed that the procedures employing acetic acid yield products which, on thin-layer chromatography (TLC), show the presence of a new substance. The new compound appeared to be an artifact, probably formed by acetylation of an aglycone. The present communication describes the characterization of this substance as aloe-emodin monoacetate, a compound hitherto unreported in the literature. UV, IR, and NMR spectra of this derivative as well as of aloe-emodin triacetate, prepared in the course of this study, are also discussed.

EXPERIMENTAL

All melting points are uncorrected. UV spectra were recorded on a spectrophotometer[1] (methanolic solutions) and IR spectra on a spectrophotometer.[2] NMR spectra were measured in $CDCl_3$ solution employing TMS as internal reference using a spectrometer.[3] Mass spectra were obtained with a single focusing mass spectrometer[4] equipped with a direct evaporator sample inlet system (MG 150). TLC was carried out on Silica Gel G (Merck) coated plates (0.30 mm.) in the solvent benzene–ethyl formate–formic acid (15:5:1) (5). The chromatogram was observed under long-wave UV lamp[5] and finally sprayed with 10% methanolic potassium hydroxide.

Preparation of Aglycones of Cascara Employing Acetic Acid (1)—Moderately fine powder of cascara bark (*Rhamnus purshiana* DC) (50 g.) was packed in a column (length, 45 cm.; i.d., 3.5 cm.) and percolated with 70% v/v methanol–water at room temperature. The eluate (2 l.) was evaporated to dryness under vacuum. To 1 g. of the dried extract was added acetic acid (10 ml.) and the mixture was refluxed on a boiling-water bath for 4 hr. After cooling, the solution was examined chromatographically (Fig. 1*a*). In a separate experiment, a mixture of the dried cascara extract (1 g.), acetic acid (10 ml.), and 25% aqueous ferric chloride solution (4 ml.) was refluxed as above. The reaction mixture, on cooling, was filtered through glass wool, concentrated under vacuum, and finally examined chromatographically (Fig. 1*b*).

Preparation of Cascara Aglycones Employing HCl—To the extract of cascara (1 g.) obtained as above was added methanol (4 ml.), water (6 ml.), and concentrated HCl (6 ml.) and the mixture was heated on a boiling-water bath for 4 hr. On cooling, the reaction mixture was extracted three times with 10-ml. portions of chloroform. The chloroform layer, after concentration under vacuum, was examined chromatographically (Fig. 1*c*).

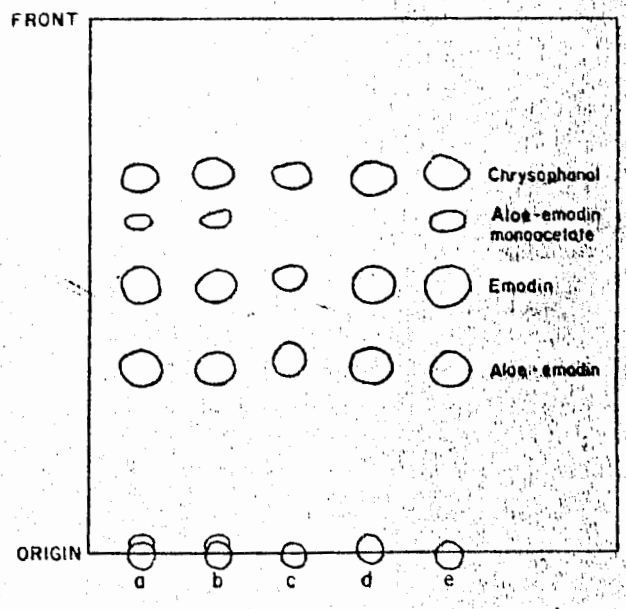

Figure 1—*Schematic thin-layer chromatogram of cascara aglycones. Solvent system, benzene–ethyl formate–formic acid (15:5:1); spray reagent, 10% methanolic KOH. Key:* a, *aglycones of cascara prepared by employing acetic acid;* b, *aglycones of cascara prepared by employing $FeCl_3$ in presence of acetic acid;* c, *aglycones of cascara prepared by employing HCl, using chloroform as the extracting solvent;* d, *aglycones of cascara prepared by employing $FeCl_3$ in presence of HCl, using chloroform as the extracting solvent;* e, *aglycones of cascara prepared by employing $FeCl_3$ in presence of HCl, using ethyl acetate as the extracting solvent.*

[1] Beckman model DK2.
[2] Perkin-Elmer model 221.
[3] Varian A-60A.
[4] Hitachi-Perkin-Elmer RMU-6D.
[5] Black Ray UVL-22.

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Table I—UV Spectra of Anthraquinones

Compd.	Assignment of Absorption Bands, mμ: Ar—C=O	Quinonoid Group
Anthraquinone	243, 252, 325	263, 272 405
1,8-Dihydroxyanthraquinone	255, 325	274, 285 430
Chrysophanol	225, 255	277.5, 287.5 430
Aloe-emodin	225, 255	274, 285 430
Quinizarin	250, 325	275 470
Quinizarin diacetate	250	265 335
Aloe-emodin monoacetate	225 (ε 24,453) 255 (ε 12,226)	274 (ε 5705) 285 (ε 5977) 430 (ε 611
Aloe-emodin triacetate	255 (ε 40,449) 322 (ε 6191)	272 (ε 14,446) 337 (ε 7429)

Preparation of Cascara Aglycones Employing Ferric Chloride and HCl (cf. 3, 4)—To the dried cascara extract (10 g.) obtained as above was added $FeCl_3 \cdot 6H_2O$ (65 g.), HCl (200 ml.), and distilled water (250 ml.), and the mixture was heated on a boiling-water bath for 4 hr. It was finally evaporated to dryness in a porcelain dish. The residue was divided in two equal portions which were extracted separately in continuous extraction apparatus with chloroform and ethyl acetate, respectively. The two extracts so obtained were separately concentrated under vacuum and examined chromatographically (Figs. 1*d* and 1*e*, respectively).

In a separate experiment, the above reaction mixture, before evaporating to dryness, was divided in two equal portions and each was separately shaken in a separator with chloroform and ethyl acetate, respectively. Each extract after the usual separation, washing, and drying, was concentrated under vacuum and was found to be chromatographically identical with the corresponding extract above.

Preparation of Aloe-Emodin (cf. 3, 4)—A mixture of powdered curaçao aloes (50 g.),[a] methanol (100 ml.), $FeCl_3 \cdot 6H_2O$ (63 g.), concentrated HCl (200 ml.), and water (250 ml.) was heated on a boiling-water bath for 6 hr. and the reaction mixture was evaporated to dryness in a porcelain dish. The residue was extracted for 24 hr. with chloroform in a continuous extraction apparatus and the extract was evaporated to dryness under vacuum. The product was fractionated on a column (200 g. silica gel powder[b]; length, 100 cm.; diameter, 2.3 cm.). Elution with benzene yielded chiefly chrysophanol, subsequent elution with benzene–ethyl acetate (9:1) gave fractions which on keeping at room temperature yielded orange-colored crystals (800 mg.), identical with an authentic sample of aloe-emodin by TLC and UV spectrum; m.p. 223–224°; mixe m.p. showed no depression.

Preparation of Aloe-Emodin Monoacetate—A mixture of alo emodin (200 mg.), ethyl acetate (10 ml.), and concentrated HC (10 drops) was refluxed on a boiling-water bath for 4 hr. and allowe to cool overnight at room temperature. A thick deposit of orang crystals was observed. The supernatant liquid was decanted an after adding 5 drops of concentrated HCl was refluxed for a furth 1.5 hr. After cooling, the reaction mixture was poured into i water (75 ml.). The resultant orange precipitate was filtered an added to the crystals obtained above and washed several times wit cold water. Repeated crystallization from ethanol and chloroform ethanol (1:3) failed to remove the unreacted aloe-emodin complet (TLC). Further purification was achieved by chromatography o silica gel column (14 g. silica gel powder; length, 40 cm.; i.d. 1 cm). Elution was done with benzene (thirty 40-ml. fractions). The first s fractions gave pure aloe-emodin monoacetate (TLC), which r crystallized from benzene as fine needles, m.p. 189–190°; mol. w (mass spectrum), 312; λ_{max}. 225 mμ (ε 24,453), λ_{max}. 255 m (ε 12,226), λ_{max}. 274 mμ (ε 5,705), λ_{max}. 285 mμ (ε 5,977), λ_{max} 430 mμ (ε 6,113); IR spectrum is shown in Fig. 2.

Preparation of Aloe-Emodin Triacetate (cf. 6, 7)—A mixture o aloe-emodin (50 mg.), anhydrous pyridine (1.5 ml.), and acet anhydride (1.5 ml.) was refluxed on a water bath (60–70°) und anhydrous conditions ($CaCl_2$ trap) for 2 hr. The reaction mixtur was allowed to stay overnight at room temperature and then was poured into ice water (100 ml.) when a yellow precipitate sep rated. The precipitate was filtered, washed several times with co water, and recrystallized once from ethanol (yield, ~75 mg.). TL examination of the sample showed the presence of several mi impurities. Chromatography on a silica gel column (20 g. silica g length, 24 cm.; i.d., 2 cm.) employing benzene and benzene–eth acetate (19:1) as eluants led to the isolation of the impurities a the pure fractions, respectively. The latter on recrystallization fro benzene gave pure aloe-emodin triacetate m.p. 177–178°; λ_{max} 255 mμ (ε 40,449), λ_{max}. 322 mμ (ε 6,191), λ_{max}. 272 mμ (ε 14,44 λ_{max}. 337 mμ (ε 7,429); IR spectrum is shown in Fig. 2.

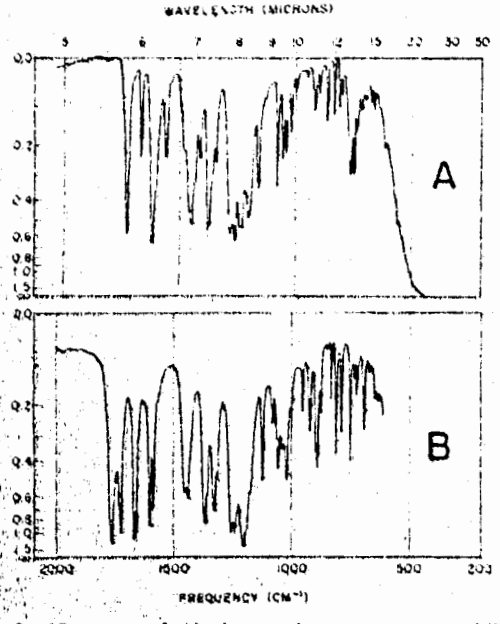


Figure 2—*IR spectra of* (A) *aloe-emodin monoacetate and* (B) *aloe emodin triacetate mulled in mineral oil.*

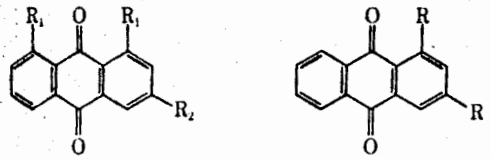
RESULTS AND DISCUSSION

Since cascara contains both *O*-glycosides and *C*-glycosides, t aglycones were prepared by treating the dried extract of the dr separately with acid as well as with acidic ferric chloride. Two se of experiments were carried out, one using acetic acid and the oth hydrochloric acid. The reaction mixture obtained with $FeCl_3$ an HCl was extracted separately with chloroform and ethyl acetat Thus five samples of aglycone mixtures were obtained. Their thi layer chromatograms are shown in Fig. 1. Chromatograms (1*a* an 1*b*) of the products prepared in presence of acetic acid exhibited characteristic spot at R_f 0.68 which was not seen in the chromat grams (1*c* and 1*d*) of the corresponding products obtained wi HCl, using chloroform as the extracting solvent. Evidently, th substance at R_f 0.68 was either a compound unstable in presence HCl or an artifact produced by reaction of acetic acid with a produ of hydrolysis. Chromatogram (1*e*) of the ethyl acetate extract of th $FeCl_3$/HCl reaction product (or of the HCl reaction product in contrast to that of chloroform extract, showed an intense spo for the above substance. This observation strongly suggested th characterization of the substance at R_f 0.68 as an artifact formed interaction of an aglycone with ethyl acetate or with acetic a formed on hydrolysis of ethyl acetate. When the chloroform ex tract of $FeCl_3$/HCl reaction product was refluxed with acetic a and a few drops of HCl, the intensity of aloe-emodin spot on t

[a] Penick.
[b] Baker.

thin-layer chromatogram of the product decreased and a new spot at R_f 0.68 was generated. Thus, the aglycone involved in the formation of the artifact was shown to be aloe-emodin (I).

For further characterization of the unknown substance, acetylation of aloe-emodin was carried out under different experimental conditions. Reaction with acetic anhydride in presence of pyridine gave the triacetyl derivative (II) (6, 7) which proved to be different from the compound under investigation on TLC examination. When aloe-emodin was refluxed with acetic acid or ethyl acetate in presence of HCl, a derivative identical with the artifact in R_f value, UV, IR, and NMR spectral data was obtained. Mol. wt. of the substance, determined by mass spectrometry, agreed with that of an aloe-emodin monoacetate. Spectral data discussed below suggested that the $—CH_2OH$ group, as expected, was acetylated in preference to the phenolic OH groups, and the compound is, therefore, the monoacetate derivative III.



I, R_1 = OH, R_2 = CH_2OH
II, R_1 = OAc, R_2 = CH_2OAc
III, R_1 = OH, R_2 = CH_2OAc
IV, R_1 = H, R_2 = H
V, R_1 = OH, R_2 = H
VI, R_1 = OH, R_2 = CH_3

VII, R = OH
VIII, R = OAc

Spectra of Aloe-Emodin and Its Mono- and Triacetyl Derivatives—*UV Spectra*—The positions and intensities of UV absorption bands of these compounds are recorded in Table I. The spectral data may be rationalized by application of the concept of partial contributing chromophores, which in the case of anthraquinones are Ar—C=O and the quinonoid moieties (8–10). The former gives rise to strong electron transfer (ET) bands at 246 mμ or higher, depending on the nature of substituents (11) and a weak $n - \pi^*$ band near 320 mμ, usually masked by more intense bands generated by further substitution. The quinonoid chromophore shows ET bands of medium and weak intensity in the region 260–276 mμ and one or more weak bands near 400 mμ due to ET and/or $n - \pi^*$ transitions.

The 400-mμ band is affected by nuclear substitutions and shifts to longer wavelengths with increase in intensity when OH groups are introduced. Alkyl substituents affect only the Ar—C=O bands, to only a small extent (11). The table also presents spectral data of anthraquinone (IV), aloe-emodin (I), and some related compounds. As expected, the spectra of 1,8-dihydroxyanthraquinone (V), 1,8-naphthanol (VI), and aloe-emodin (I) are closely similar. This analytical approach was successfully applied to the interpretation of UV spectra of acetyl derivatives of aloe-emodin obtained in this study. Acetylation of the $—CH_2OH$ group should not affect the spectrum of aloe-emodin. Acetylation of the nuclear OH groups, on the other hand, would be expected to shift the 400 mμ band to shorter wavelengths—see, for example, spectral data for quinizarin (VII) and quinizarin diacetate (VIII) (12). The aloe-emodin monoacetate, exhibited the same UV spectrum as aloe-emodin. Thus the CH_2OH group involved in acetylation is the $—CH_2OH$ group. The triacetyl derivative, as expected, shows a major hypsochromic shift of the 400 mμ band to 337 mμ.

IR Spectra—The characteristic features of these spectra are the bands generated by quinonoid and acetoxyl carbonyl groups. The IR spectrum of aloe-emodin two C=O stretching frequencies are observed at 1,674 and 1,626 $cm.^{-1}$. These are assigned to the free and the bonded carbonyl groups respectively (13). Aloe-emodin monoacetate exhibits the two quinonoid carbonyl frequencies in essentially the same positions *viz.*, 1,670 and 1,620 $cm.^{-1}$ (Fig. 2*a*). This shows that the bonded character of the C-9 carbonyl group remained unchanged during acetylation. The acetyl group must, therefore, be located in the side chain at C-3. In the triacetate, chelation of the carbonyl is not possible, and the 1,620 $cm.^{-1}$ band moves to a higher frequency—*viz.*, 1,600 $cm.^{-1}$ (Fig. 2*b*). The C=O stretching band of $—CH_2OAc$ in the mono- and the triacetate appears at 1,730 $cm.^{-1}$, while the phenolic acetate groups at C-1 and C-8 in the triacetate absorb, as expected (14), at higher frequencies *viz.*—1,760 and 1,770 $cm.^{-1}$

NMR Spectra—Characterization of the aloe-emodin monoacetate was readily achieved by NMR. The two phenolic protons which give signals in the spectrum of aloe-emodin at 12.01 and 11.96 δ are seen in that of the monoacetate at 12.08 and 12.05 δ. In addition, the monoacetate shows the CH_3 protons of the acetoxy-methyl group at 2.19 δ. In the spectrum of the triacetate, the signals for the OH protons are absent and the corresponding acetoxy protons generate a signal at 2.45 δ, the CH_3 protons of the acetoxy methyl group being at 2.18 δ.

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