Some Coumarin Constituents of *Prunus mahaleb* L. Fruit Kernels V

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Abstract □ It was possible to isolate 2-glucosyloxy-4-methoxyethyl *trans*-cinnamate from the alcoholic extract of the defatted *Prunus mahaleb* L. fruit kernels. The structure of this compound was confirmed by IR, NMR, and mass spectra. Further evidence was sought through its de-ethylation into 2-glucosyloxy-4-methoxy *trans*-cinnamic acid which was identical in every respect with authentic material prepared synthetically. Through acetylation it afforded a tetraacetate, the structure of which was also confirmed by NMR and mass spectra. The identity of the sugar as glucose was ascertained by enzymatic and acid hydrolysis and subsequent paper chromatography in three different solvent systems.

Keyphrases □ Coumarins—*Prunus mahaleb* fruit kernels □ Paper chromatography—separation, identification □ Optical rotation—identity □ IR spectrophotometry—structure □ NMR spectroscopy—structure □ Mass spectroscopy—structure

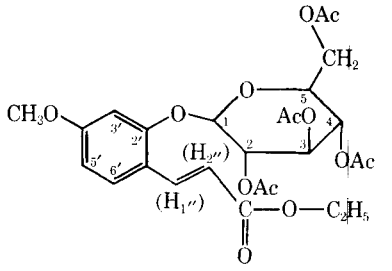
In a previous communication (1), it was reported that *Prunus mahaleb* L. kernels contain herniarin (7-methoxycoumarin) both in a free form and as a glucoside. The present author, during examination of the alkaloidal fraction of the kernels, was able to isolate a crystalline compound which appears not to have been reported before. Although this compound was isolated from the alkaloidal fraction, it was found to be free of nitrogen and gave negative color reactions for alkaloids. The aim of this work was to study the structure of this compound. Pharmacological studies on this compound have been carried out and will appear shortly.

EXPERIMENTAL

Melting points were determined on a Kofler block and are uncorrected. IR spectra were determined in KBr using a Leitz Unicam S.P. 200 G. NMR spectra were determined in DMSO using a 60-Mc. instrument. Mass spectra were determined with CH_4, instrument at 70°, using an electron energy of 70 eV. Optical rotations were determined with a Perkin-Elmer 141 polarimeter.

Isolation of 2-Glucosyloxy-4-methoxyethyl *trans*-Cinnamate (I)—Five hundred grams dried and defatted mahaleb kernels was extracted with 95% ethanol to exhaustion. After concentration of the alcoholic extract, ether was added to precipitate choline and other glycosides. After decantation of ether, the precipitate was dissolved once more in alcohol and ether was added once again. The combined ethereal extracts were concentrated and extracted with diluted HCl to remove the alkaloids. The aqueous acidic solution was then made alkaline with ammonia and extracted with chloroform. The chloroform was dried over anhydrous sodium sulfate and then concentrated. After evaporation of chloroform, a yellow semisolid residue with a strong characteristic odor was left. Repeated crystallization from chloroform–methanol gave colorless needles, m.p. 180–182°, $[\alpha]_D^{22} = -58.8°$ (in pyridine, yield 90 mg. about 0.2% of alcoholic extract, C = 56.22%; H = 6.30%; $C_{18}H_{24}O_9$ requires C = 56.24% and H = 6.29%). It gave a single spot on silica gel G developed with chloroform–methanol (85:15; R_f, 0.267) which could be seen as a dark blue fluorescent spot in UV light. It gave a positive reaction for Molisch test and negative reactions for alkaloids and flavonoids.

The UV spectrum in ethanol showed maxima at λ 239, λ 296, and λ 322 mμ ($\epsilon = 8.83 \times 10^3$, 1.23×10^4, and 1.55×10^4, respectively). The IR spectrum showed bands characteristic for hydroxy groups (3380 cm.$^{-1}$), carbonyl in conjugation with a double bond (1680 and 1615 cm.$^{-1}$), 1,2,4-trisubstituted aromatic structure (1570, 772, 766, and 760 cm.$^{-1}$) (2). This compound was identified as 2-glucosyloxy-4-methoxyethyl *trans*-cinnamate (Structure I).



2-glucosyloxy-4-methoxyethyl *trans*-cinnamate(acetate)

I

The mass spectrum of this compound (Fig. 1) showed a molecular ion peak at *m/e* 384 (46.7%) from which the expulsion of methanol gave a peak at *m/e* 352 (8.5%). The important fragmentation behavior of the molecule includes the following features (Scheme I); expulsion of 162 mass units by loss of $C_6H_{10}O_5$ for the glucose moiety leads to an ion at *m/e* 222 (22.3%) formulated as "A." Subsequent expulsion of ethanol by the possible mechanism indicated by the arrows leads to the highly conjugated ion "B" which appears as the strongest ion in the spectrum (base peak). The latter ion loses carbon monoxide presumably from the cyclohexadienone system with the formation of ion "C" which appears at *m/e* 148 (81.5%). Further degradation of the latter ion happens by expulsion of methyl radical giving the stabilized ozonium ion "D" at *m/e* 133 (58.7%). Subsequent expulsion of carbon monoxide gives a $C_7H_5O^+$ ion at *m/e* 105 (11.7%) which may be represented by the constituent "E."

Enzymatic Hydrolysis of the Glucoside—Twenty milligrams of Compound I was dissolved in about 20 ml. of water, about 20 mg. of B-glucosidase in 20 ml. water was added, one drop of toluene for preservation, and the mixture was kept for about 10 hr. at 37° with occasional shaking. The mixture was then extracted with ether. The ethereal layer was dried over anhydrous sodium sulfate and then examined chromatographically on paper (Whatman No. 1) using the upper phase of *n*-butanol–acetic acid–water (4:1:5) for developing. Spots were located under UV light. The liberated aglycone gave one spot (R_f, 0.863) and was different from the original compound (R_f, 0.79) and from herniarin (R_f, 0.905).

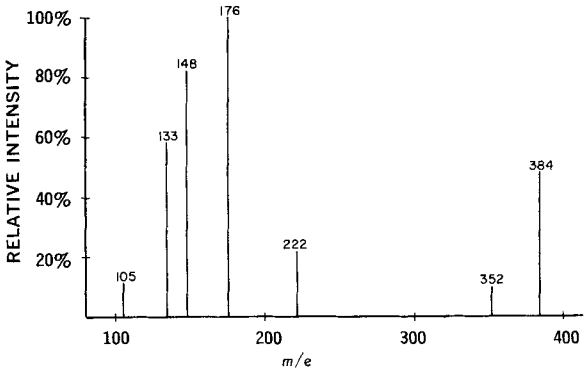
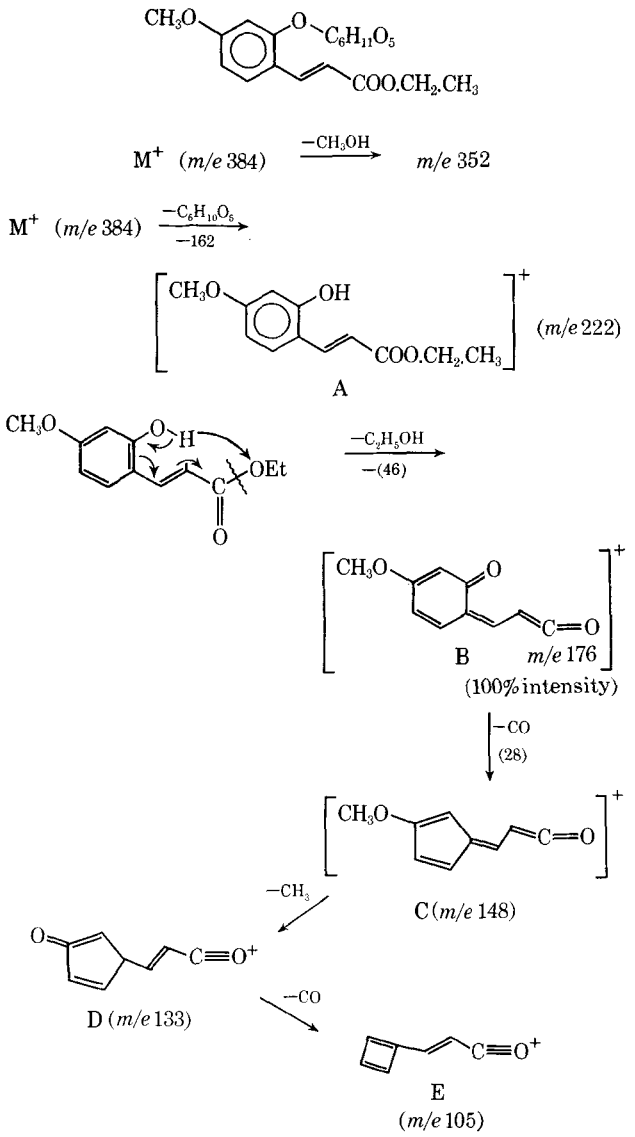


Figure 1—*Mass spectrum of glucoside.*



Scheme I—Fragmentation pattern of glucoside.

The aqueous layer after filtration and concentration under reduced pressure was tested chromatographically on paper (Whatman No. 1) against reference sugars using the following solvent systems: (*a*) *n*-butanol–acetic acid–water, 4:1:5 (3); (*b*) ethyl acetate–pyridine–water, 5:2:5 (4); and (*c*) collidine saturated with water (5).

The chromatograms were developed for about 24 hr. (descending). After drying at room temperature, they were sprayed with *p*-anisidine–phosphoric acid reagent (6). In the three solvent systems, the aqueous phase gave a brown spot which was identical to that of glucose run as a reference material.

Acid Hydrolysis of the Glucoside—To the alcoholic solution (20 ml.) of about 20 mg. of the glucoside, about 20 ml. 2 *N* sulfuric acid was added and the mixture was refluxed for about 1 hr. The reaction mixture was then diluted with water and extracted with ether. The ethereal layer was tested chromatographically on paper (Whatman No. 1, solvent: upper phase of *n*-butanol–acetic acid–water, 4:1:5) and on silica gel G (solvent: benzene–3% methanol). It gave one spot identical to herniarin.

The aqueous layer, after being neutralized with barium carbonate, was passed through a column of ion-exchange resin (Dowex 50WX, 50–100 mesh, hydrogen form) to remove any barium ions and then concentrated under reduced pressure and tested chromatographically on paper using the same solvents mentioned previously. The only brown spot was identical to reference glucose.

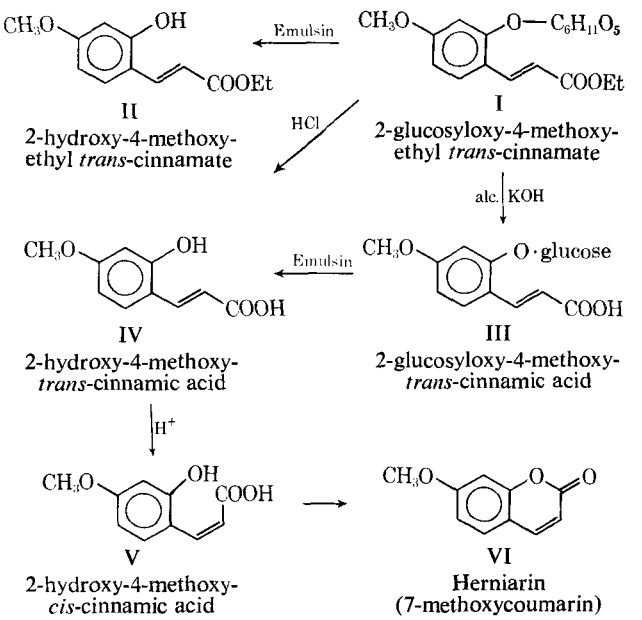
Acetylation of the Glucoside—One hundred milligrams of the crystalline compound was dissolved in 1 ml. of anhydrous pyridine, then 1 ml. of acetic anhydride was added and the mixture was heated at 100° for about 2 hr. The pyridine and acetic anhydride were evaporated under reduced pressure leaving a colorless crystalline mass. Recrystallization from aqueous methanol gave colorless needles, m.p. 160–162°, $[\alpha]_D^{22} = -56.5°$ (in chloroform). Tested chromatographically on silica gel G, it gave one fluorescent spot (solvent: chloroform–methanol, 85:15; R_f, 0.35). The IR spectrum in KBr showed the disappearance of the hydroxyl band. The NMR spectrum showed three acetyl groups at $\delta = 2.17$ and another one at $\delta = 2.14$ accounting for the acetyl group attached to C_6 of the sugar moiety (Structure I), one methoxy group at $\delta = 4$. There were bands indicating the presence of an ethyl group at $\delta = 1.42$ (triplet, $J = 7$ c.p.s.) and $\delta = 4.3$. It showed also three aromatic protons: H(3′) at $\delta = 6.98$ (singlet), H(5′) at $\delta = 6.9$ (doublet, $J = 9$ c.p.s.), and H(6′) at $\delta = 7.9$ (doublet, $J = 9$ c.p.s.). Two *trans*-ethylenic protons appeared: H(1″) at $\delta = 7.91$ (doublet, $J = 16$ c.p.s.) and H(2″) at $\delta = 6.55$ (doublet, $J = 16$ c.p.s.). The sugar moiety appeared as a complex multiplet ($\delta = 5$–6).

The mass spectrum showed the presence of a molecular ion at *m/e* 552. Further fragmentation showed the loss of four molecules of acetic acid successively and then behaved like the mother compound.

Deethylation of the Glucoside—Twenty-five milligrams of the crystalline glucoside was dissolved in about 25 ml. aqueous alcohol (20%), then 25 ml. of 1% KOH in aqueous alcohol was added and the mixture was left overnight at room temperature. The next day the solution was passed through a column of ion-exchange resin (Dowex 50WX, 50–100 mesh, hydrogen form) to neutralize the potassium hydroxide. The effluent neutral solution was evaporated under reduced pressure. The colorless mass left, when crystallized from water, gave plates m.p. 196–198°, $[\alpha]_D^{22} = -50.7°$ (in pyridine) (12 mg. yield); reported for 2-glucosyloxy-4-methoxy *trans*-cinnamic acid (III), 194–196° (7). When tested chromatographically on paper (Whatman No. 1, solvent: *n*-butanol–acetic acid–water, 4:1:5), it gave one spot (R_f, 0.635) which was identical with authentic 2-glucosyl-4-methoxy *trans*-cinnamic acid prepared synthetically (7). IR spectra of both products were also identical.

DISCUSSION

2-Glucosyl-4-methoxyethyl *trans*-cinnamate (I, Scheme II) was isolated from the alkaloidal fraction of the alcoholic extract of *Prunus mahaleb* L. kernels. NMR spectra of the compound and its tetraacetate showed signals for an ethyl group. These signals were found to be persistent even after thorough drying or using solvents other than ethanol or methanol for crystallization. The presence of the ethyl group as a part of the molecule and not as impurity was proved by mass spectra of both the compound and its acetate. That the compound occurs in the *trans*-form was shown by the IR spectrum [985 cm.$^{-1}$ (2)] and by the NMR spectra showing the presence of two *trans*-ethylenic protons, H(1″) at $\delta = 7.91$ (doublet, $J = 16$ c.p.s.) and H(2″) at $\delta = 6.55$ (doublet, $J = 16$ c.p.s.).



Scheme II—Reactions of the glucoside.

Nevertheless, on acid hydrolysis the compound gave herniarin. The formation of herniarin may be accounted for by the transformation of the liberated 2-hydroxy-4-methoxy-*trans*-cinnamic acid (IV, Scheme II) to the *cis*-form in presence of hydrogen ions. However, enzymatic hydrolysis did not give herniarin but gave 2-hydroxy-4-methoxyethyl *trans*-cinnamate (IV) which was found to be less mobile on paper than herniarin. The structure of the glucoside (I) was also ascertained through its de-ethylation to the free-acid glucoside (III) which was identical in every respect with authentic material prepared synthetically (7).

SUMMARY

2-Glucosyloxy-4-methoxyethyl *trans*-cinnamate was isolated in a crystalline form from the alcoholic extract of *Prunus mahaleb* L. fruit kernels. The structure of this compound was proved by IR, NMR, and mass spectra. It was further ascertained by de-ethylation of the compound to the free-acid glucoside which was identical with authentic material. On acetylation, it afforded a tetraacetate, the spectral studies of which (IR, NMR, and mass spectra) assured the suggested structure. On acid and enzymatic hydrolysis, it afforded glucose which was identified by chromatographic techniques.

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Tablet-to-Tablet Variation of Drug Content of Sugar-Coated Tablets Containing Drug in the Sugar Coat

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Abstract □ In dosage forms where active drug is added to a sugar coat, it may be shown that the distribution of assays should be normal and the standard deviation should be proportional to the square root of the number of coats. Although, in a series of batches, the standard deviation was found linearly related to the square root of the number of coats, the plot failed to intersect at the origin and did not have the required slope, presumably due to secondary contributions to the variation such as pan build-up.

Keyphrases □ Drug content variation—sugar coating, tablets □ Tablet coatings—drug content variation □ Core size, tablets—coating drug content variation □ Coatings, tablet—drug content relationship

In recent years, several publications have dealt with tablet-to-tablet variation of uncoated tablets or compression coated tablets. Garrett (1) and Garrett and Olson (2) have studied the problem from the point of view of content of drug; Brochmann-Hanssen and Medina (3), Smith *et al.* (4), Lazarus and Lachman (5), and Airth *et al.* (6) analyzed the situation from the point of view of weight variation. Some publications, *e.g.*, those of Lachman *et al.* (7) and Kaplan (8), conclude that statistical variation may be used as a means of evaluating processes; whereas others, *e.g.*, those by Grundman and Ecanow (9) and French *et al.* (10), have been concerned with the problem of statistical sampling.

The inherent variations in sugar-coated tablets have been touched upon by Bhatia (11), but this paper aims not at the statistical variation to be expected but rather on the effect of incompatibilities. Anderson and Sakr (12), in a comprehensive treatment of the statistics of sugar coating, studied the mean line average as a parameter, since smoothness of the tablet was their main point of discussion. Since some coated tablets contain the active component in the sugar coat, it would appear important to know whether expected statistical variations might apply in such a situation. Mattocks (13) has pointed out the problem associated with uniformity of coating, and Butensky (14) has found that, weightwise, the coefficient of variation rises to a maximum of 7% at a stage prior to the final subcoating. The work by Anderson and Sakr (12) also implies that the coefficient of variation of the mean line average appears to level off at a certain stage of the coating operation,

Table I—Tablet-to-Tablet Variation of Sugar-Coated Tablets, Showing Standard Deviation as a Function of Number of Coats, *n*

Number of Coats (*n*)	$\sqrt{n}$	$10^2 \times SD$ (Σ), mg.	Average Drug Content per Tablet ($n\mu$), mg.	$\sqrt{n\mu}$
1	1.00	1.6	0.120	0.347
2	1.41	3.0	0.244	0.494
6	2.45	12.5	0.693	0.835
11	3.32	19.3	1.214	1.102
13	3.61	23.6	1.456	1.208
16	4.00	22.5	1.790	1.340