methanol, there remained 22 g. of undistillable material, presumably glycol (70.5%).

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Infrared Absorption of Some Steroid Digitonides

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Although formation of stable molecular compounds (digitonides) from the saponin, digitonin, and steroids possessing a C_3 hydroxyl group is known to be ultimately dependent upon a 3β-orientation of the latter group,[1] little information is available regarding the nature of the secondary valence forces responsible for complex formation. In particular, the extent to which, in polyfunctional molecules, groups other than the 3β-hydroxyl group may be involved, through hydrogen bonding or other associative phenomena, has not been ascertained. The work reported in this communication was undertaken in order to examine the effects of digitonide formation upon the characteristic infrared absorption frequencies of carbonyl groups located at various positions in the steroid nucleus. 3β-Hydroxy steroids possessing carbonyl functions at positions 6, 7, 11, 12, 17 and 20 were investigated in this connection. Two α,β-unsaturated ketonic derivatives were also included. Owing to insolubility of the digitonides and to the possibility of dissociation in solution, measurements were made on suspensions of the crystalline complexes in mineral oil. Absorption data for the free sterols in mineral oil and in carbon disulfide solution are listed for comparison (Table I).

TABLE I

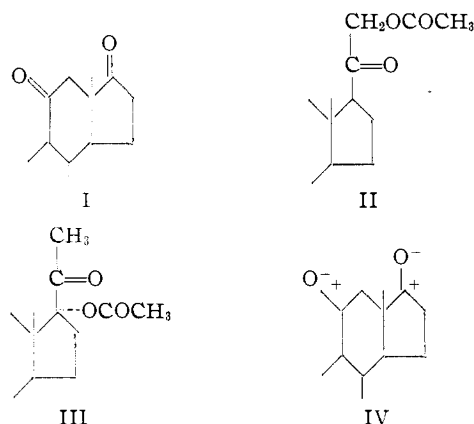
INFRARED ABSORPTION OF STEROID DIGITONIDES

No.	Compound*	Maxima, cm.$^{-1}$ (Mineral oil) Sterol	Digitonide	(CS$_2$ soln.) Sterol
1	6-Ketocholestanol	1692	1704	1715
2	7-Ketocholestanol[a]	1691	1700	1715
3	11-Ketoergostenol[b]	1693	1704	1709
4	Hecogenin[c]	1688	1697	1712
5	Δ^5-Androstene-3β-ol-17-one	1720	1735	1742
6	Δ^5-Pregnene-3β-ol-20-one	1673	1697	1709
7	Δ^4-Cholestene-3β-ol-6-one[a]	1666	1677	1692
8	7-Ketocholesterol[a]	1660	1675	1675

* Compounds indicated by lettered superscripts were furnished through the kindness of the following investigators: (a) L. F. Fieser, Harvard University, Cambridge, Mass. (sample no. 7 represents a highly purified preparation, m.p. 155.5–156.5°), (b) M. Tishler, Merck and Co., Rahway, N. J., (c) G. Mueller, University of Tennessee, Nashville, Tenn.

From inspection of the data (columns 1 and 2) it is clear that digitonide formation is accompanied by small, but significant, displacements (8 to 24 cm.$^{-1}$) of the carbonyl stretching maxima toward higher frequencies in all cases investigated. These displacements correspond to *increases* in the force constants of the carbon–oxygen double bonds. Since shifts to lower frequencies, associated with *decreases* in the C=O force constants, result from hydrogen bonding involving carbonyl groups,[2,3] participation of forces of this kind in stabilization of the digitonides would appear to be minimized.

High frequency displacements of the C=O stretching maxima have been observed by Jones and his collaborators[2,4] in a number of instances in which two appropriately oriented carbonyl groups occupy proximate positions in space. 11,17-Diketo steroids (I) and ketol acetates of types II and III[5] may be cited as illustrative of the several recorded examples of this phenomenon. Suppression



of polar resonance structures (*cf.* IV) by mutual dipole repulsions, with consequent reinforcement of the double bond character of the respective carbonyl groups, may account for these observations.[6] However, the assumption that similar processes (for example, interactions between carbonyl groups of different molecules) are involved in the case of the digitonides, requires acceptance of a number of elaborate hypotheses.

A more plausible explanation for the spectral shifts encountered in the present work is based upon consideration of forces operative within the steroid crystals. The results of X-ray analyses indicate that hydrogen bonding, particularly that involving the C_3 hydroxyl group, plays an important part in determining the lattice structure of these substances.[7] Thus sterols possessing a single hydroxyl group (C_3) are doubled in the unit cell by head-to-head association of the hydroxyl functions

(1) A discussion of digitonide formation is given by L. F. Fieser and M. Fieser, "Natural Products Related to Phenanthrene," 3rd ed., Reinhold Publ. Corp., New York, N. Y., 1949, pp. 102, 648.

(2) R. N. Jones, P. Humphries, F. Herling and K. Dobriner, THIS JOURNAL, **74**, 2820 (1952).

(3) R. S. Rasmussen, D. D. Tunnicliff and R. R. Brattain, *ibid.*, **71**, 1068 (1949); M. St. C. Flett, *J. Chem. Soc.*, 1441 (1948); M. M. Davies and G. B. B. M. Sutherland, *J. Chem. Phys.*, **6**, 755 (1938); W. Gordy, THIS JOURNAL, **60**, 605 (1938).

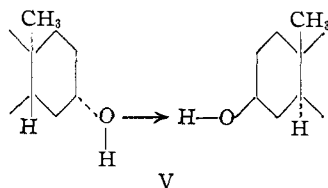
(4) R. N. Jones and K. Dobriner, *Vitamins and Hormones*, **7**, 293 (1949).

(5) R. B. Turner, THIS JOURNAL, **75**, 3489 (1953).

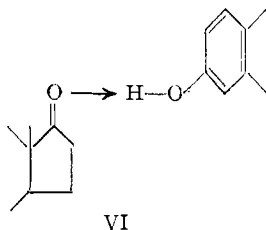
(6) *Cf.* A. D. Walsh, *Trans. Faraday Soc.*, **43**, 1158 (1947); R. N. Jones, D. A. Ramsay, F. Herling and K. Dobriner, THIS JOURNAL, **74**, 2828 (1952).

(7) D. Crowfoot, *Vitamins and Hormones*, **2**, 439 (1944).

of two contiguous molecules (*cf.* V). Substances, which, like estrone, possess hydroxylic and ketonic groups at opposite ends of the molecule are ordinarily propagated in the crystal by head to tail association as in VI. Disruption of these forces in solution by intervention of solvent molecules re-



V



VI

sults in the expected shifts of carbonyl absorption in the direction of higher frequencies (Table I, column 3). The suggestion may be made that analogous frequency displacements in the digitonides are attributable to insulation, by the indifferent hydrocarbon nucleus of the digitonin molecule, of centers normally engaged in hydrogen bonding, with the further possibility of coördination of the 3β-hydroxyl groups with the glycosidic portion of the saponin.

Ultraviolet absorption of 7-ketocholesterol (no. 8) and of the corresponding digitonide in alcohol solution has also been investigated. Both substances show maximum absorption at 237.5 mμ with molar extinction coefficients of 14,000 and 12,600, respectively. Interpretation of these results is unfortunately complicated by the possibility of dissociation of the digitonide in the dilute solution employed.

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Isolation and Identification of Quercetin and Some Quercetin Glycosides from Plums (*Prunus Salicina*)

BY BYRON L. WILLIAMS AND SIMON H. WENDER

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This paper reports an extension of a series of studies of the flavonols and their glycosides present in fruits. Williams, *et al.*, have previously isolated and identified quercetin (3,3',4',5,7-pentahydroxyflavone) and isoquercitrin (quercetin-3-glucoside) from grapes,[1] black currants[2] and apricots.[3] This paper reports the isolation and identification of quercetin, isoquercitrin, quercitrin (quercetin-3-rhamnoside) and a quercetin-3-arabinoside from yellow plums.

(1) B. L. Williams and S. H. Wender, THIS JOURNAL, **74**, 4372 (1952).
(2) B. L. Williams, C. H. Ice and S. H. Wender, *ibid.*, **74**, 4566 (1952).
(3) B. L. Williams and S. H. Wender, *Arch. Biochem. Biophys.*, **43**, 319 (1953).

Experimental

Fifty pounds of ripe California yellow plums, *Prunus Salicina*, of the 1952 season were processed through a wet grinder with the whole fruit including the seeds and skins being ground. After 2 hours extraction with 20 gal. of distilled water at boiling temperature, the extract was filtered, then treated on an ion exchange column and later on a Magnesol column by the procedures previously used by Williams and Wender[3] for apricots. Flavonoid material was adsorbed at the top of the Magnesol column giving a zone about 40 mm. wide. The chromatogram was developed with ethyl acetate saturated with water. A zone about 30 mm. wide, yellow under ultraviolet light but not detectable in the visible, moved off the column first. The contents of this zone were found to be non-flavonoid and so were not studied further. The next zone, which will be called flavonoid fraction I, was yellow in the visible and also yellow under ultraviolet light, and its major component was later identified as quercetin. The next zone eluted, flavonoid fraction II, moved very close to the first. It was brown under ultraviolet light and yellow in the visible, and its flavonoid compound was later identified as quercetin-3-arabinoside.

The next zone eluted off the column (flavonoid fraction III) was brown in ultraviolet light and yellow in the visible, and was later shown to be a mixture of quercitrin and of a quercetin glucoside not identical with either isoquercitrin or quercimeritrin (quercetin-7-glucoside). The glucoside of fraction III has not been definitely identified as yet. The next eluted fraction (IV) was yellow in the visible and red-brown under ultraviolet light and its flavonoid was later identified as isoquercitrin. Each of the four fractions was recovered from the ethyl acetate solution by concentrating the eluate *in vacuo* to about 3 ml. and adding 10 ml. of pentane, followed by centrifugation and decantation.

Identification of Quercetin.—The quercetin zone eluted from the column was further purified as follows: The impure solid was dissolved in 10 ml. of ethyl alcohol, put on paper sheets (20 × 60 cm.) of Whatman No. 1 paper, chromatographed in 60% acetic acid–water and then air-dried for 4 hr. The resulting yellow zone on each paper at an R_f of about 0.40 was cut out and the pigment eluted with 95% ethyl alcohol in an air-tight chamber. The combined eluate from 25 sheets was concentrated to 0.5 ml. and then 10 ml. of boiling water was added. The yellow solid was separated, crystallized and identified as quercetin by the methods described in previous studies by Ice and Wender[4]; yield approximately 50 mg.

Identification of Quercetin-3-arabinoside.—The solid from fraction II was purified and then identified as quercetin-3-arabinoside by procedures similar to those reported for the isolation of this compound from the leaves of *Vaccinium myrtillus* by Ice and Wender.[4] The highly purified crystals had a m.p. 214–216°; approximate yield 16 mg.

Identification of Quercitrin.—The solid from flavonoid fraction III from the column was dissolved in 15 ml. of ethyl alcohol and put on paper strips in the manner described in previous paragraphs. The strips were first chromatographed using 15% acetic acid–water. The yellow zone at R_f about 0.6 (approximately 5 cm. wide) was cut out and eluted. Inasmuch as this zone was more dispersed than usual for the quantity recovered, a sugar analysis was made. Three mg. of the solid was hydrolyzed by refluxing for 2 hr. with 2% sulfuric acid, then the filtrate was neutralized and chromatographed as described under fraction II. By this procedure, both rhamnose and glucose were found to be present. Since the known quercetin disaccharides do not readily move on a Magnesol adsorption column with wet ethyl acetate as the chromatographing agent, and zone III did, this would indicate that zone III was most likely a mixture of a quercetin glucoside and a quercetin rhamnoside. This solid was, therefore, dissolved in ethyl alcohol once more and again put on fresh paper strips and chromatographed in 5% acetic acid–water. Each strip was loaded with 0.5 mg. of solid as before, but this time the strips were allowed to run for 14 hours. This brought the forward end of the zone to the lower end of the strip. The zone was about 10 cm. wide. The strips were allowed to air dry 3 hours, then a strip 1 cm. wide was cut out of the center of this zone and discarded. The forward part of the zone was eluted with ethyl alcohol in a leaching chamber, the alcohol evaporated, and the solid recrystallized as described under

(4) C. H. Ice and S. H. Wender, THIS JOURNAL, **75**, 50 (1953).