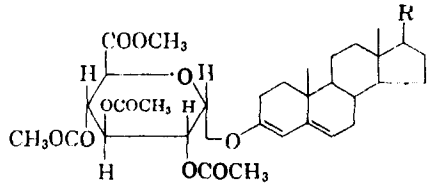
α,β-unsaturated ketone vibrations are known to absorb, concomitant with the appearance of two bands at 1655 and 1631 cm.$^{-1}$, known to be associated with conjugated -C=C- stretching vibrations.

Further evidence for the diene structure was obtained from the formation of the brown color of the polyacetylated derivatives of the three enol-glucosiduronates with tetranitromethane[26] (Table II) as well as the bathochromic shift of the ultraviolet absorption maximum from 240 mμ for the parent steroid to 237.5 mμ for the derivatives.

Compounds XV, XVII and XIX can be written as



XV, R = O
XVII, R = $-COCH_3$
XIX, R = $-CH(CH_3)(CH_2)_3CH(CH_3)_2$

The enol-glucosiduronates are assumed to be $\Delta^{3,5}$-dienes since they are the only heteroannular dienes that can be written for these substances. A homoannular diene system can be expected to have an ultraviolet absorption maximum near 275 mμ.[27] This is further substantiated by the increase of levo-rotation of these compounds. Callow and Young[28] have shown that introduction of a 5,6-double bond causes a strong increase in levo-rotation while the introduction of a 4,5-double bond causes a marked increase in dextrorotation.

The appearance of a purple color with Zimmermann's reagent also shows retention of the 17-ketone in XV, while a brownish coloration with XVII indicates a C-20 ketone. Compound XIX produced no color with this reagent due to the loss of its ketone group.

The acetylated methyl ester derivative of androst-4-ene-3,17-dione shows a bathochromic shift of 2.5 mμ which compares favorably to the shift produced for cholesta-3,5-dien-3-yl-acetate (from 240 to 238 mμ).[27] When compound XV was subjected to methanolysis (XVI), the ultraviolet absorption maximum underwent a hypsochromic shift of 2.5 to 240 mμ. The infrared characteristics of this substance were comparable to that of compound XV with respect to its absorption near 1655 and 1631 cm.$^{-1}$ and its lack of absorption near 1675 cm.$^{-1}$. Retention of the enol-glucosidic linkage was further borne out by the fact that on reacetylation of XVI the ultraviolet absorption maximum again showed a bathochromic shift to 237.5 mμ. This phenomenon may be caused by the inability of the acetylated glucuronic acid moiety to approach the diene system, while the deacetylated sugar residue may be able to approach more closely and exert a modifying influence on the electronic spectrum of this compound.

Compound XVIII was assigned the structure of a glucuronolactone, rather than the free acid, on the basis of its non-acidic properties, relative insolubility, elemental analysis and the infrared absorption spectrum.

The ultraviolet spectrum and optical rotation of this substance was not recorded because of its insolubility in the proper solvents.

Acknowledgments.—The authors wish to express their gratitude to Dr. Preston Perlman of Schering Corporation, Dr. Juan Berlin of Syntex S. A., Dr. Elmer Alpert of Merck and Co. and Dr. Kenneth W. Thompson of Organon, Inc., for their generous contributions of steroids used for this study.

BOSTON 18, MASS.

(26) A. Werner, *Ber.*, **42**, 4324 (1909).
(27) U. Westphal, *ibid.*, **70**, 2128 (1937).
(28) R. K. Callow and F. G. Young, *Proc. Roy. Soc.* (*London*), **A157**, 194 (1936).

[CONTRIBUTION FROM THE DEPARTMENTS OF BIOCHEMISTRY AND MEDICINE, BOSTON UNIVERSITY SCHOOL OF MEDICINE]

D-Glucopyranosiduronates. II. Infrared Absorption Spectra of Some Methyl-[steroidyl-2,3,4-tri-*O*-acetyl-β-D-glucosid]-uronates[1]

BY ERIKA SMAKULA, JEHAUDAH H. LEFTIN AND HERBERT H. WOTIZ

RECEIVED FEBRUARY 7, 1958

Eleven steroid glucosiduronates were investigated in the amorphous and crystal states. These two methods yielded complementary information in the difficult interpretation of the superficially simple yet complex spectra of these partially flexible molecules. The spectra uniformly showed bands of proportionally high intensity due to the sugar moiety common to all compounds and chiefly arising from its four ester groups. The spectra were differentiated by a low intensity proportion of bands arising from the functional groups of the parent steroids. Qualitative intensity evaluation of these bands permitted an estimation of the steroid/sugar ratio. The stretching vibrations of the glucosidic linkage were shown to be characteristically perturbed by the environmental influence of neighboring steroid bands.

Steroid glucosiduronates are large molecules flexible at the substituent ester linkages of the sugar residue and at the glucosidic linkage. The degree of freedom of rotation about the C–O–C bonds of the three equatorial acetoxy groups and the C–C–O linkage of the methyl ester group can be expected to be relatively high and nearly identical for all compounds. Since the glucosidic linkage connects two bulky molecular residues, steric barriers to free rotation are likely to exist.

(1) These studies have been supported by research grants and an Institutional Research Grant from the American Cancer Society, research grants from the National Institutes of Health and from Aids for Cancer Research (Boston, Mass.).

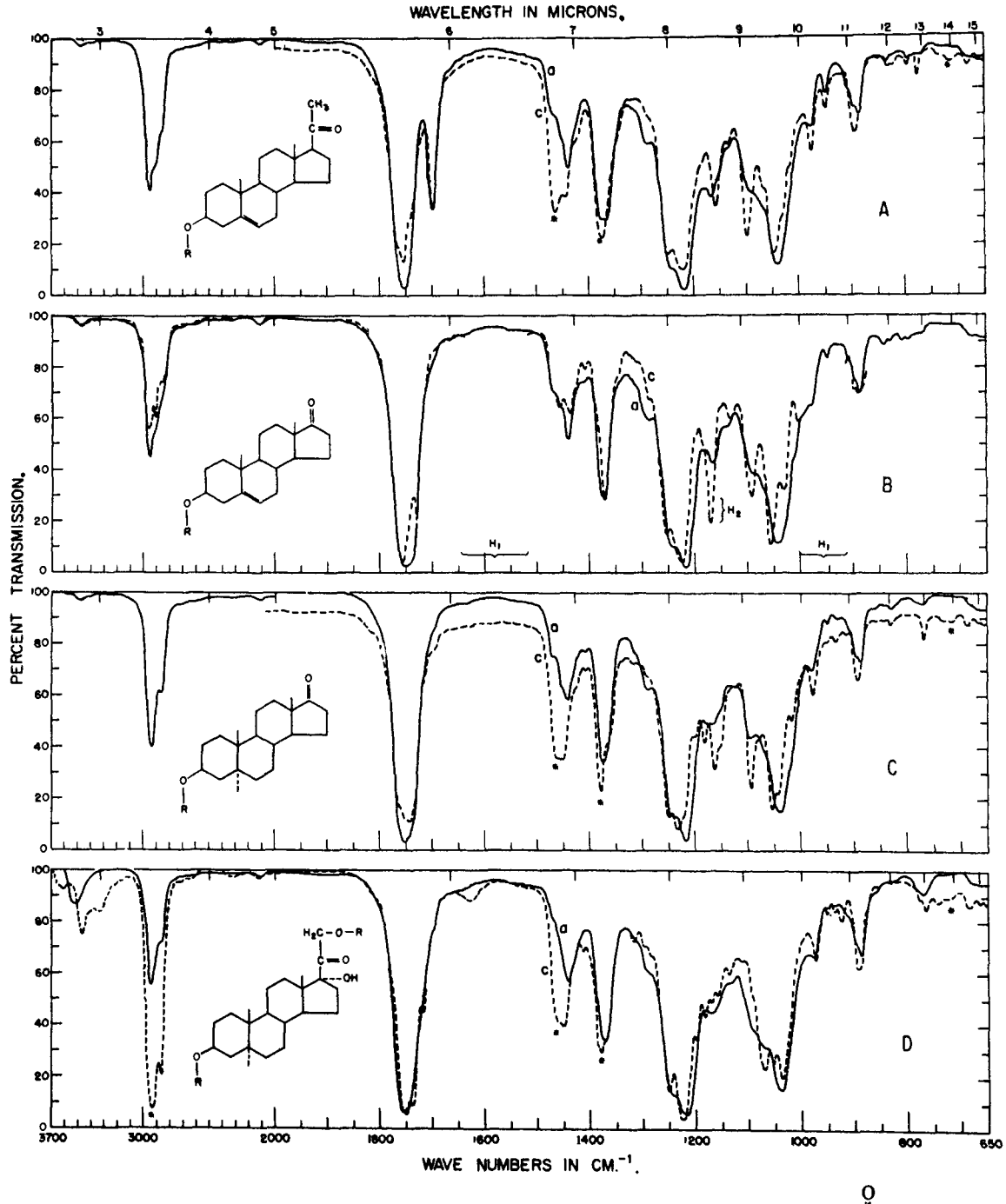
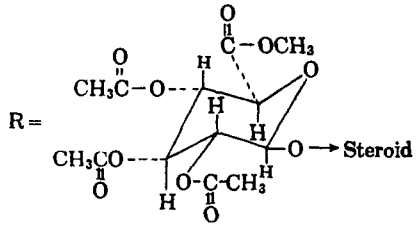


Fig. 1.———— (a), amorphous phase spectra, obtained from glassy melts of compounds A, B, C, D; ----------(c), crystal spectra obtained from Nujol mulls for compounds A, C, D, and from a hexachlorobutadiene mull for compound B. Asterisks mark superimposed Nujol bands. H_1 marks regions of omitted hexachlorobutadiene absorption. H_2 gives approximate intensity contribution by a hexachlorobutadiene band.



They are expected to differ depending on the position of the glucoside on the steroid nucleus and the respective intramolecular environment.

All spectra may be expected to show similar features in the absorption characteristics of their common sugar residue, to differ with respect to the differing steroid residue and to show a variation bssociated with modified vibrations of the C–O–C aonds of the glucosidic linkage.

Infrared investigations were carried out in the crystalline and the amorphous phase on all glucosiduronates. The spectra produced by these two

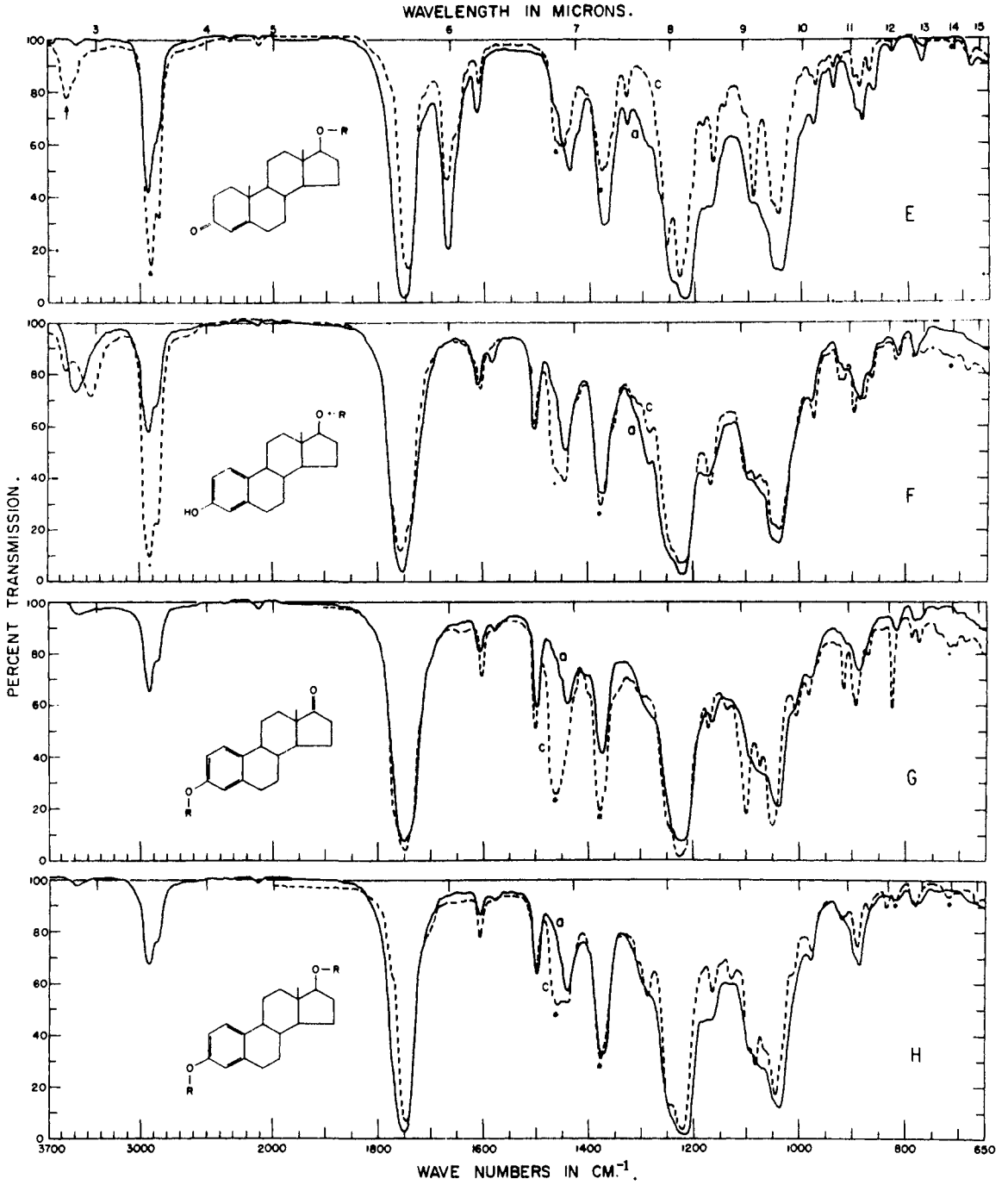


Fig. 2 —R = same as in Fig. 1. ———— (a). amorphous phase spectra obtained from glassy melts of compounds E, F, G, H; ---------- (c), spectra of the crystalline state obtained from nujol mulls for compounds E, F, G, H. Asterisks mark superimposed nujol bands In E the arrow refers to the O–H stretching vibration arising from ethanol of crystallization.

states were shown to be of complementary value in their interpretation; the former were much more specific for the individual glucosiduronates compared to the striking similarity of the amorphous phase spectra. The chief difference between the various amorphous phase spectra appeared in a window region of the dominating sugar moiety from about 1700 to 1500 cm.$^{-1}$, where some steroidal functional groups absorb. Crystal spectra facilitated the recognition of steroidal ketone bands due to their separation from the strong summation band of the four ester C=O groups of the sugar residue. The detailed structure in the finger print region provided good differentiation between different compounds, except for the hazards of polymorphism.[2] The absence of the discriminating influences of combined inter- and intra-molecular effects such as polymorphism and selective rotational isomerism is necessary for a direct comparison of related compounds. The conversion of

(2) E. Smakula, A. Gori and H. H. Wotiz, *Spectrochim. Acta*, **9**, 346 (1957).

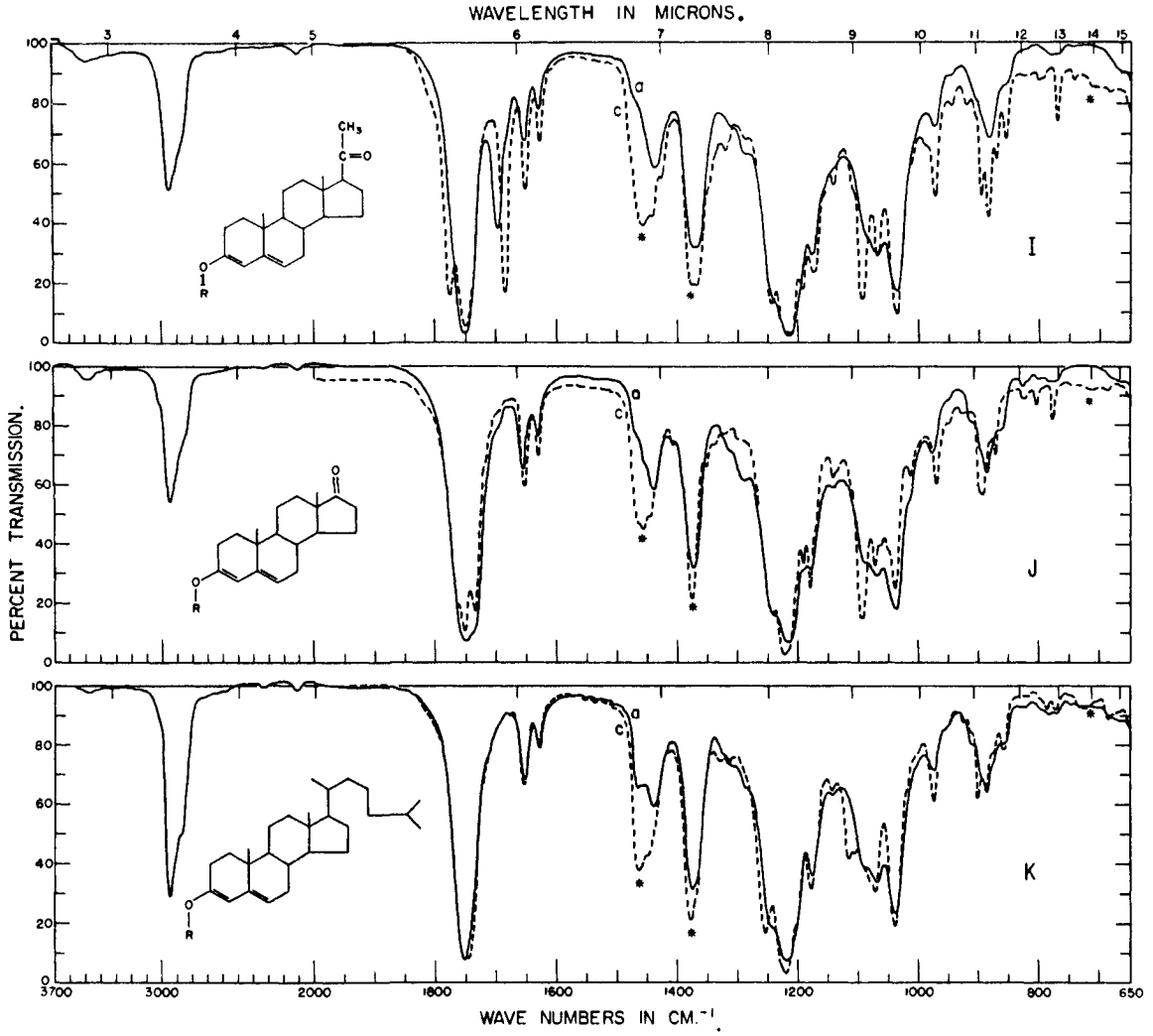


Fig. 3.—R = same as in Fig. 1. ———— (a), amorphous phase spectra obtained from glassy melts of Compounds I, J and K; ---------- (c), crystalline spectra obtained from nujol mulls of Compounds I, J and K. Asterisks mark superimposed Nujol bands.

all substances into the amorphous phase rendered conditions suitable for the detection of the position of the glucosidic linkage and the determination of the steroid/sugar ratio.

The infrared absorption spectra of the methyl polyacetylglucopyranosiduronates of the following steroids were investigated: (A) Δ^5-pregnene-3β-ol-20-one; (B) Δ^5-androstene-3β-ol-17-one; (C) androstane-3β-ol-17-one; (D) allopregnane-3β,17α,21-triol-20-one (bis-derivative); (E) Δ^4-androstene-17β-ol-3-one; (F) $\Delta^{1,3,5}$-estratriene-3,17β-diol (mono-derivative); (G) $\Delta^{1,3,5}$-estratriene-3-ol-17-one; (H) $\Delta^{1,3,5}$-estratriene-3,17β-diol (bis-derivative); (I) $\Delta^{3,5}$-pregnadiene-3-ol-20-one; (J) $\Delta^{3,5}$-androstadiene-3-ol-17-one; (K) $\Delta^{3,5}$-cholestadiene-3-ol.[3]

Experimental

Infrared investigations were carried out with a Perkin-Elmer model 21 double beam spectrophotometer with a sodium chloride prism using slit program 927. The spectra of all steroid glucosiduronates presented in Fig. 1, 2 and 3 were recorded in the crystalline state as mulls (Nujol and hexachlorobutadiene) and in the amorphous phase as glassy melts. No decomposition occurred on melting of these compounds which could be demonstrated in each case by crystallization of the original compound from the amorphous melt. Several compounds were found to exist in at least two different crystal modifications.

Results and Discussion

The difference between spectra of the crystalline and the amorphous state arises partly from effects associated with the flexibility of molecules which are capable of exhibiting free rotation or rotational isomers about some of their bonds and partly from the influence of intermolecular forces on positions and intensities of intramolecular vibrations.[4,5]

Rotational isomers are likely to exist selectively in the crystal state due to the steric confinement of the molecules in a crystal lattice. They are most probably a major cause of polymorphism and are likely to exist in increased numbers in the amorphous phase (which is comparable to the liquid

(3) H. H. Wotiz, E. Smakula, N. N. Lichtin and J. H. Leftin, THIS JOURNAL, 81, 1704 (1959).

(4) N. Sheppard, "Molecular Spectroscopy" (The Institute of Petroleum), 1955, p. 136.

(5) R. S. Halford, *Ann. N. Y. Acad. Sci.*, 69, 63 (1957).

state), their number being restricted only by steric barriers to free rotation.

The slight differences in energy of such rotational isomers will influence the position and intensity of the absorption frequencies of the bonds involved. The co-existence of different molecular species will produce a summation spectrum. The structural detail of the crystal spectra of the steroid glucosiduronates appears to represent a selection of a low number of molecular species; the artificial simplicity as well as the broad, unresolved, and highly unsymmetrical band complexes of the amorphous phase spectra appear to be due to a summation of individual spectra produced by many closely absorbing molecular species. Since in the amorphous phase the molecules are disordered, collision broadening will be a further cause for overlapping and merging of bands. In contrast, a further differentiating effect on the crystal spectra of the compounds and their polymorphous modifications is caused by the influence of intermolecular forces in a crystal lattice on the positions and intensities of intramolecular vibrations and by the narrower band width. The spectra are discussed according to the expected contribution of the sugar residue, the steroid residue and the glucosidic linkage.

The Sugar Moiety.—A striking feature of all the spectra is the overwhelming proportional intensity of the absorption characteristics of the sugar residue with its four ester substituents. The ester bands associated with C=O and coupled C–O and C–C stretching vibrations are intramolecular summation bands of closely absorbing components with respect to each molecular species. They are extramolecular summation bands with respect to co-existing rotational isomers. The bands near 1750 and 1220 cm.$^{-1}$ do not exhibit much difference in the crystalline and amorphous phase except for a better resolution of the C=O ester stretching vibration from the steroidal ketone in the crystal spectra. The latter also show slight shifts of the absorption maximum by selective intensity enhancement of one component, and a better resolution of a broad shoulder near 1250 cm.$^{-1}$ of the very broad and intense ester C–O band complex. The latter band is sensitive to orientation. At least four components could be brought out under favorable conditions of sample preparation. The identical band positions in all amorphous phase spectra and their assignments are given in Table I.

The Steroid Residue.—The characteristic steroid absorption frequencies and assignments for the crystal and amorphous phase spectra are given in Table II. The crystal spectra with their sharper bands and better resolution were found to be more suitable for the recognition of the identifying bands, except in Compounds C and G where the crystal structure is not favorable to the resolution of the closely absorbing 17-ketone and ester C=O vibrations as in Compounds B and J. For intensity comparisons the amorphous phase is preferable. All compounds showed the required diagnostic absorption characteristics of the respective steroid residue in agreement with the known or expected band positions and intensity proportions, the latter being reduced in the two bis-glucosiduronates C and H. The intensities of steroid bands in the finger print region—below 1350 cm.$^{-1}$—are proportionally much smaller than the intensities of steroidal ketone groups.[6,7] Since the latter are of low intensity compared to the fourfold ester C=O band of the sugar residue, the contribution of the former is negligible but detectable in suitable regions of the spectrum (Table II).

Initial values of integrated absorption intensities of the C=O stretching vibration are known to vary with their molecular environment. Jones[8] gives values of 3.65 units (unit = 10^4 moles^{-1} × l. × cm.$^{-2}$)[9] for the Δ^4-3-keto group; 2.69 units for the 17-keto group; 1.79 units for the 20-keto group in steroids.

TABLE I

THE SUGAR RESIDUE

Band positions in the amorphous phase spectra identical for all compounds. Frequencies in cm.$^{-1}$. V = very, S = strong, M = medium, W = weak, sh = shoulder.

	Band position	Assignment	Ref.
[a]	1753 v.s.	C=O stretch, ester	11
[b]	1440 m.	Deformation of carbomethoxy methyl	12
[c]	1373 m.s.	Symmetric deformation of methyl in the ester	11
	1290 w.		
[d]	1245 sh.	Coupled vibrations C–O and	13
	1218 v.s.	C–C stretching for ester	13
[e]	1040 s.	Coupled vibration C–O and C–C stretching for ester	13
	980–975 m.		
[f]	887 m.	Complex band; maximum due to deformation of C-1 C-H β-configuration	14, 15
	775 v.w.	Ring breathing vibration	14, 15

[a] This frequency has an overtone near 3480 cm.$^{-1}$ in all spectra. The high frequency branch of this broad band is identical for all spectra. The low frequency branch merges in the individual spectra with the respective steroidal ketone bands. [b] This absorption peak is the strongest component of a band complex due to methyl and methylene deformation vibrations of the acetoxy groups and the steroid. [c] Steroid methyl groups contribute to this band on the low frequency side. [d] The band complex is less resolved near 1235 cm.$^{-1}$ for the aromatic compounds G and H and the enol-glucosiduronates I, J, and K, probably due to a contribution of a =C–O vibration of the glucosidic linkage on carbon 3. [e] This absorption maximum is the strongest component of an extremely broad and asymmetric band envelope, which is expected to contain other absorption frequencies associated with the ester groups, sugar ring stretching vibrations and vibrations associated with the glycosidic linkage. [f] Other components of this complex are probably due to the acetate group. [g] The 887 cm.$^{-1}$ band together with the very weak g band supports the β-configuration of the glucosiduronate.

(6) R. N. Jones and C. Sandorfy, "Techniques of Organic Chemistry," Vol. IX, A. Weissberger, ed., Interscience Publishers, Inc., New York, N. Y., 1956, Chapter IV, p. 446.

(7) R. N. Jones, B. Nolin and G. J. Roberts, THIS JOURNAL, 77, 6331 (1955).

(8) Ref. 6, p. 465.

(9) The values for acetate and methyl ester C=O vibrations in steroids are given as 3.24 and 3.13. Those for sugar esters are not available. They are not expected to vary greatly from those above. Using these values the expected reduction of steroid C=O intensities would be in fractions of the fourfold ester C=O intensity: about 1/3.5 in E; 1/5 in B,C,G, and J; 1/7 in A and I; and 1/14 in the bis-glucosiduronate D.

TABLE II: DIAGNOSTIC BANDS OF THE STEROID RESIDUE, CRYSTAL AND AMORPHOUS PHASE: cr. = crystal, am. = amorphous, ~ = about, sh. = shoulder, n.r. = not resolved.

Band positions of steroid residue																						Assignments of vibrations	Ref.
A		B		C		D		E		F		G		H		I		J		K			
cr.	am.	cr.	am.	cr.	am.	cr.	am.	cr.	am.	cr.	am.	cr.	am.	cr.	am.	cr.	am.	cr.	am.	cr.	am.		
						3620																OH stretching	11
						3465	3530	3560[a]	..	3550	3470												
						3330				3360													
1697	1699	1726																					
			n.r.[b]	n.r.	n.r.[b]	1717	n.r.[b]	1671	1668			n.r.[b]	n.r.[b]			1685	1698	1736	n.r.			C=O stretching	16
				1740[b]		~1700sh.													~1740[b]				
								1611	1613							1652	1654	1653	1656	1652	1657	Conjugated C=C stretching	11, 16
																1628	1630	1629	1631	1628	1632		
										1607	1616	1603	1607	1607	1607							Aromatic ring skeletal vibrations	11
											1583		1580		1577								
										1505	1503	1501	1499	1500	1498								
		1408	1408	1408	1408	1414	1414		1422				1408					1407	~1408			CH_2 bending perturbed by neighborhood of C=O[c]	10
	1367															1368	n.r.					C 21 methyl bending	10
								1330	1328													Finger print bands in Δ^4-3 ketosteroid[c]	12
								942	940														
								685	682														
			830	829	830													826	828			C-16 methylene rocking[c]	7
	795		795					873	865	822	817	827	820	819	818	873	~865	872	~866	858	~865	CH out of plane bending[c]	11, 12, 17
										788	785	791	785		785	858	n.r.		n.r.		n.r.		

All spectra refer to the methyl, polyacetyl glucosiduronates of the following steroids: (A) Δ^5-pregnen-3β-ol-20-one; (B) Δ^5-androsten-3β-ol-17-one; (C) androstan-3β-ol-17-one; (D) allopregnane-3β,17α,21-triol-20-one (bis-glucosiduronate); (E) Δ^4-androsten-17β-ol-3-one; (F) $\Delta^{1,3,5}$-estratriene-3,17β-diol (mono-glucosiduronate); (G) $\Delta^{1,3,5}$-estratrien-3-ol-17-one; (H) $\Delta^{1,3,5}$-estratriene-3,17β-diol (bis-glucosiduronate); (I) $\Delta^{3,5}$-pregnadien-3-ol-20-one; (J) $\Delta^{3,5}$-androstadien-3-ol-17-one; (K) $\Delta^{3,5}$-cholestadien-3-ol. [a] O–H stretching band due to ethanol of crystallization. This band is not present in the amorphous melt, due to evaporation of trapped ethanol. It is also lost on crystallization from an amorphous melt causing a band shift from 1090 to 1099 cm.$^{-1}$ and from 1043 to 1052 cm.$^{-1}$. This shift is believed to be due to the unbonding of an ethanolic O–H from the oxygen of the glucosidic linkage: R–OH...O<C. [b] Band causes broadening of the low frequency branch of the ester C=O stretching band. [c] These bands are all very weak.

Although integrated intensity determinations were not carried out, the above sequence of decreasing intensities, relative to that of the ester and C–H stretching bands (adjusted in the various spectra to nearly the same intensity), is evident for the isolated Δ^4-3-ketone band in E at 1668 cm.$^{-1}$ and the isolated 20-ketone band in A and I at 1699 cm.$^{-1}$ with 20%, respectively, ~35% transmission. The overlapping 17-ketone band evades evaluation.

The intensity contribution of the 17-ketone stretching vibration in Compounds B,C,G and J was established as an asymmetric broadening of the absorption peak and an increase of band width on the low frequency side of the 1750 cm.$^{-1}$ band by superposition of the amorphous phase spectra of these substances with those of Compounds A and E which have an isolated symmetric ester band. The presence of the 17-ketone group was further confirmed in each case by a band at 1408 cm.$^{-1}$ which is characteristic for a CH_2 bending vibration specifically perturbed by the neighborhood of the 17 ketone group.[10] Compound D was designated a bis-glucosiduronate because of the low intensity of the 20-ketone band, which prevents its resolution in the amorphous phase, and because of the reduced intensity of the C–H stretching band near 2900 cm.$^{-1}$. Compounds A and C, adjusted to approximately the same intensity for the sugar residue absorption bands, were used as reference compounds.

Compounds H and F were identified as the bis- and C-17-mono-glucosiduronates, respectively, by intensity adjustment of the ester bands for both compounds to approximately the same strength (Fig. 2), where H showed a reduced intensity of the C–H stretching absorption and no O–H stretching band. The absence of the O–H stretching vibration is expected for the bis-glucosiduronate. Compound F, the mono-glucosiduronate, exhibited an O–H stretching absorption band as well as a band near 1055 cm.$^{-1}$, characteristic for attachment of the glucosidic linkage to the five-membered ring (Fig. 4), which is distinctly different from the absorption characteristics for the attachment to the aromatic ring of the estrone derivative G. In the enol-glucosiduronates I, J and K (Fig. 3) the replacement of the conjugated ketone by the heteroannular diene system is strikingly exhibited by two absorption bands near 1655 and 1631 cm.$^{-1}$.

The Glucosidic Linkage.—Absorption of appreciable intensity is expected to arise from stretching vibrations associated with the C–O–C linkage near 1100 cm.$^{-1}$. The crystal spectra indeed show

(10) R. N. Jones, A. R. H. Cole and B. Nolin, THIS JOURNAL, **74**, 5662 (1952).

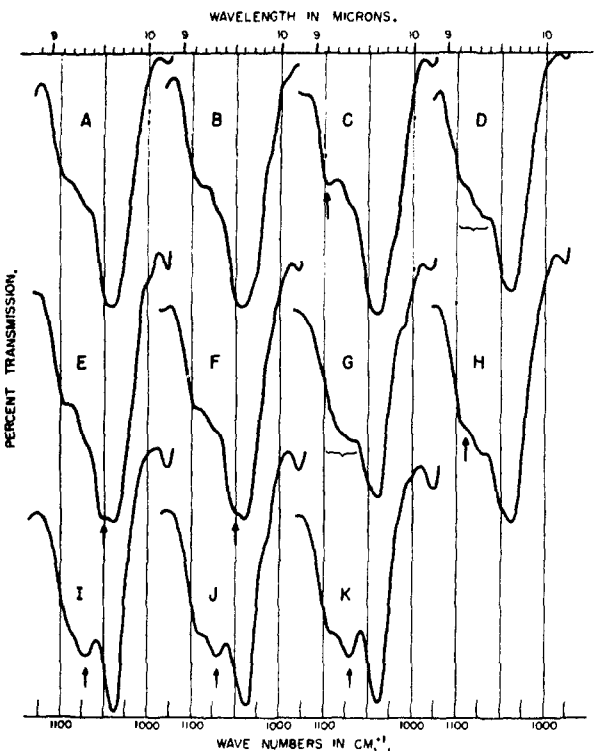


Fig. 4.—Influence of the glucosidic linkage on the shape of the band complex in the region 1100–1000 cm.$^{-1}$.

Cpds. A and B	ether linkage at C-3 in a 3β-hydroxy-Δ^5-steroid
C	ether linkage at C-3 in a 3β-hydroxy-5α-steroid
D	ether linkage at C-21 and C-3 in a 3β-hydroxy-5α-steroid.
E and F	ether linkage at C-17,
G	ether linkage at C-3 in an aromatic ring A steroid
H	ether linkage at C-17 and C-3 in an aromatic ring A steroid
I,J,K	ether linkage at C-3 in a 3-hydroxy-$\Delta^{3,5}$-diene steroid

Arrows and brackets point out regions of differentiation.

several strong bands in this region, where the detail in structure is likely to be associated with a selection of stereoisomers about the glucosidic linkage. In the amorphous phase spectra the region 1175–1000 cm.$^{-1}$ is extremely ill-defined. However, a major contribution to the broad band complex can be expected to arise from this linkage in the form of summation bands of rotational isomers (see also e, Table I).

As a skeletal vibration an ether linkage is known to be extremely sensitive to environmental influences due to interaction with neighboring bonds which may alter absorption frequencies.[11] The environment of the glucosidic linkage is different in the various compounds on the side of the attachment to the steroid, thus it is expected to modify the C–O–C vibrations. Barriers to free rotation about the C–O–C linkage will vary most probably according to the position on the steroid nucleus or the steroid side chain. They can also be expected to have a discriminating influence on the vibrations involved. Although these perturbations are small, they give rise to an appreciable and reproducible intensity difference in the band contour from 1125–1000 cm.$^{-1}$. These changes are not due to a contribution of steroid fingerprint bands, pointed out to be negligible above. Nevertheless, the detection of the modifying influence of environment on the stretching vibrations of the C–O–C linkage can also be based on the dissimilarity of band contours in the three 17-ketosteroid derivatives B, C and J and the two C-21 steroid glucosiduronates A and I, since the background absorption—except for the modified vibrations of the glucosidic linkage—can be expected to be nearly the same in each group of compounds.

Band contours in the region 1125–1000 cm.$^{-1}$ are given in Fig. 4 for all compounds. Those with the same environment near the glucosidic linkage show nearly identical band contours such as the two C-3 conjugated Δ^5-steroid glucosiduronates, the two C-17 conjugated glucosiduronates and the three C-3 conjugated $\Delta^{3,5}$-glucosiduronates. Contours A and B were taken as reference for the differences described. The band contours of the C-3 conjugated glucosiduronates of the two Δ^5-steroids are very nearly the same on superposition of their spectra, which were adjusted to approximately the same concentration. This near identity eliminates the possibility of a noticeable contribution due to the characteristic fingerprint bands of a 17-ketosteroid.[12] The 20-ketone group does not induce absorption of significant intensity in this region.[12]

Compounds B and C are well suited for comparison, their differences being confined to the neighborhood of the glucosidic linkage. In compound C the band contour of the C-3 conjugated 5α-steroid glucosiduronate shows a resolved absorption peak at 1097 cm.$^{-1}$ and a more pronounced shoulder at 1020 cm.$^{-1}$.

In compound D the band contour of the side chain glucosiduronate appears as an increase in intensity between 1095 and 1060 cm.$^{-1}$, superimposed on that of the C-3 conjugated 5α-steroid glucosiduronate. The band contour of the two C-17 glucosiduronates E and F is distinguished from that of the C-3 Δ^5-glucosiduronates by an intensity increase and an extra peak near 1050 cm.$^{-1}$. Neither the Δ^4-3-ketone group[12] nor the aromatic ring gives rise to a significant absorption in this region.

A marked change of the intensity contribution to the band complex can be expected for the C-3 conjugated aromatic glucosidic linkage and for the C-3 conjugated enol-glucosidic linkage. The at-

(11) L. J. Bellamy, "The Infrared Spectra of Complex Molecules," London, Methuen and Co., Ltd., 1954, p. 466.

(12) Ref. 6, p. 505.

(13) Ref. 6, p. 503.

(14) S. A. Barker, E. J. Bourne, M. Stacey and D. H. Whiffen, *J. Chem. Soc.*, 171 (1954).

(15) S. A. Barker, E. J. Bourne, R. Stephens and D. H. Whiffen, *ibid.*, 3468 (1954).

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

(17) H. Hirschmann, THIS JOURNAL, **74**, 5357 (1952).

tachment of an ether linkage to a double bond is known to cause a high frequency shift toward 1250 cm.$^{-1}$ of one of the stretching vibrations associated with this linkage.[9]

The band contour of the aromatic C-3 conjugated glucosiduronate G is clearly distinguished by a relative increase in intensity and a comparative lack of resolution between 1100 and 1065 cm.$^{-1}$. The superposition of this band contour with that of the C-17 conjugated Compound H is evident from a better resolution near 1090 cm.$^{-1}$.

The strikingly similar band contour of the enol-glucosiduronates I, J and K shows also a relative intensity increase near 1075 cm.$^{-1}$. The band complex is distinguished by the resolution of an absorption peak at 1070 cm.$^{-1}$ and by narrowing of the major absorption band at 1038 cm.$^{-1}$ both of which appear to be due to a loss of band components near 1025 and 1050 cm.$^{-1}$.

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[CONTRIBUTION FROM THE DEPARTMENT OF AGRICULTURAL BIOCHEMISTRY, UNIVERSITY OF MINNESOTA]

Constitutional Studies on the Glucomannan of Konjak Flour[1]

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Methylation of the glucomannan isolated from the bulbs of *Amorphophallus konjac* yields the corresponding methylated derivative which gives upon hydrolysis a mixture of the 2,3,4,6-tetramethyl ethers of D-glucose and D-mannose (1 mole), the 2,3,6-trimethyl ethers of D-glucose and D-mannose (11 moles) and the 2,6-dimethyl ethers of D-glucose and D-mannose (1 mole). These data are in accord with the results of periodate oxidation of the polysaccharide. The structural significance of the results is discussed.

Amorphophallus konjac C. Koch (*Syn. Conophallus konjak* Schott), a member of the family *Araceae* is the source of the so-called "konjak flour" a popular article of food in Japan. The bulbs of three year old plants are cut into thin slices which are then dried and powdered to give konjak flour.

Preliminary studies[2-6] on the konjak flour showed that the polysaccharide was composed of glucose and mannose residues but results differed with regard to the relative proportions of the two component sugars.

Degradation of the konjak polysaccharide by a sporulating bacterium isolated from konjak flour was reported[3] to produce a trisaccharide, "laevidulin" ($[\alpha]_D$ −11.5°), and a similar trisaccharide, "laevidulinose" ($[\alpha]_D$ −15°), composed of D-mannose (2 parts) and D-glucose (1 part), was obtained[6] by the action of Takadiastase on konjak flour. However, the structure of these oligosaccharides was not established. Acetolysis[7] of the konjak glucomannan followed by saponification afforded a trisaccharide ($[\alpha]_D$ −16° in water) which was shown to be composed of mannose (2 moles) and glucose (1 mole). The structure of this oligosaccharide, which appeared to be identical with laevidulin or laevidulinose, was not established. Hydrolysis of methylated konjak mannan was also reported[7] to give a mixture of 2,3,4-tri-*O*-methyl-D-glucose, 2,3,4,-tri-*O*-methyl-D-mannose and 2,3,6-tri-*O*-methyl-D-mannose and based on these results a formula for the polysaccharide was advanced. Since the proof of the structure of the components of the hydrolyzate of the methylated glucomannan did not appear to be entirely satisfactory, it seemed desirable to re-examine the constitution of konjak glucomannan using the more modern techniques that were not available to the earlier workers. This paper is concerned with the results of methylation and periodate oxidation studies on a glucomannan isolated from a commercial sample of konjak flour.

The glucomannan was isolated from konjak flour by precipitation from aqueous solution as the copper complex following the procedure adopted for the Iles glucomannan.[8] The polysaccharide was regenerated from the copper complex by adding dilute hydrochloric acid, the last traces of copper being removed by means of Versene (ethylenediaminetetraacetic acid). Konjak glucomannan was obtained as a white powder which showed $[\alpha]_D$ −38° in water and which upon hydrolysis gave rise to a mixture of D-glucose and D-mannose in a molar ratio of 2:3.

Methylation of the glucomannan first with methyl sulfate and alkali, and then with silver oxide and methyl iodide yielded the fully methylated polysaccharide which showed $[\alpha]_D$ −19° in chloroform. Fractional precipitation of the methylated polymer from a solution in acetone with ether and petroleum ether indicated that the substance was essentially homogeneous. Upon methanolysis followed by hydrolysis, the methylated glucomannan gave a mixture of methylated sugars which were shown by column chromatographic analysis[9] to consist of 2,3,4,6-tetra-*O*-methyl-D-glucose, 2,3,4,6-

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