Tetrahedron. Vol. 26, pp. 1943 to 1951. Pergamon Press 1970. Printed in Great Britain

NEW COMPOUNDS FROM AVOCADO PEAR—II

Y. KASHMAN, I. NÉEMAN and A. LIFSHITZ
Departments of Chemistry and Biochemistry, Tel-Aviv University, Tel-Aviv, Israel

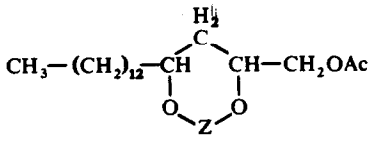
(*Received in the UK* 1 *December* 1969; *Accepted for publication* 16 *December* 1969)

Abstract—The configuration of some of the new compounds reported previously,[1] to be isolated from avocado has been elucidated by synthesising the carbonate, acetonide and sulfites of one of the compounds. The characterisation of several other compounds of the same group is reported.

IN A previous communication we reported the isolation and structural determination of a new class of compounds found in the seeds and flesh of avocado.

Compound I $HC{\equiv}C{-}(CH_2)_{11}{-}\overset{4}{C}HOH{-}\overset{3}{C}H_2{-}\overset{2}{C}HOH{-}\overset{1}{C}H_2OAc$

is typical to this class of long chain aliphatic compounds. The other compounds in this class vary in the unsaturation at the ω-carbon of the chain and in the degree of oxidation at the other end. The molecule of compound I contains two asymmetric centers at C-2 and C-4. We now report experiments carried out for the determination of the stereochemistry of these two asymmetric centers. For this purpose, the two hydroxyls at C-1 and C-4 were fixed in space, by synthesising the cyclic carbonate, acetonide and the sulfites.



III: Z, C=O
IVb: Z, $C(CH_3)_2$
V: Z, S=O(ax.)
VI: Z, S=O(eq.)

The carbonate was synthesized by treating compound II, the fully hydrogenated derivative of compound I, with $COCl_2$. The IR spectrum clearly showed the absence of the two hydroxyls and the formation of a carbonate ester (1736 cm^{-1}). In the NMR spectrum, the previously known signals at high field appeared: δ 0·87 (3H), CH_3; δ 1·28-$(CH_2)_{-12}$; δ 2·10 (3H)-$OCOCH_3$ and δ 1·86 (2H) attributed to $H_3H_{3'}$. The two C-1 protons were also visible at δ 4·10; (these appeared as an 8 line pattern—the AB part of an ABX system with C-2 proton), but the H-2 and H-4 protons, with which we were concerned were indistinguishable in the very complex multiplet between δ 4·2 and δ 4·9 ppm. Attempts to simplify this pattern by hydrolysing the acetate group at C-1, or by using different solvents for the NMR spectra were unsuccessful, so that no equivocal conclusion could be obtained from the carbonate ester.

The acetonide (IVb) was next synthesized by boiling compound II with acetone in the presence of anhydrous $CuSO_4$. The appearance in the NMR spectrum of two singlets, attributed to the two Me groups, at δ 1·40s (3H) and δ 1·44s (3H), ascertained

formation of the acetonide, but no further information could be obtained from the low field area. All of the four protons that resonate in this range appeared together as a complex multiplet between δ 3·60 and δ 4·30 ppm.

In an attempt to simplify this complex multiplet, we treated the acetonide with alkali in order to hydrolyse the acetate group on C-1 and convert it into the hydroxy acetonide (IVa). In the NMR spectrum of the last compound, the C-1 protons appeared at δ 3·53 (as an 8 line pattern—the AB part of an ABX system with C-2-H). This shift was expected as it is known that protons adjacent to a primary alcohol appear in the NMR spectrum 0·3–0·5 ppm higher than those adjacent to a primary acetate.[2] However, no change was observed in the signal of the protons on C-2 and C-4. Again, they appeared together as a very complex multiplet at δ 3·90 and, as in the case of the carbonate ester, no conclusion could be drawn as to the stereochemistry of the compound.

The best way to change the magnetic anisotropy around C-2 would be to oxidize the primary alcohol. Unfortunately, all the various basic oxidative methods employed failed to produce the desired compound.

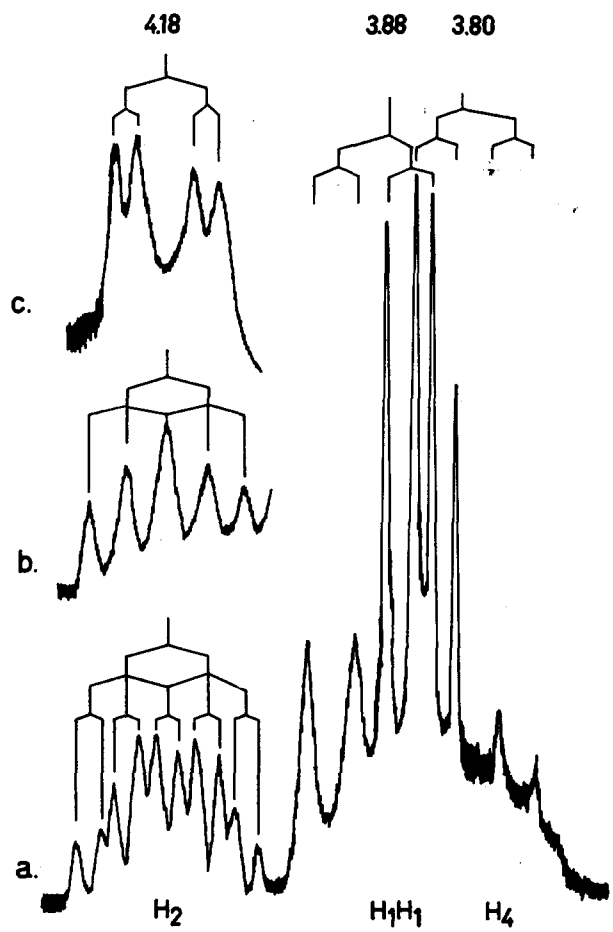
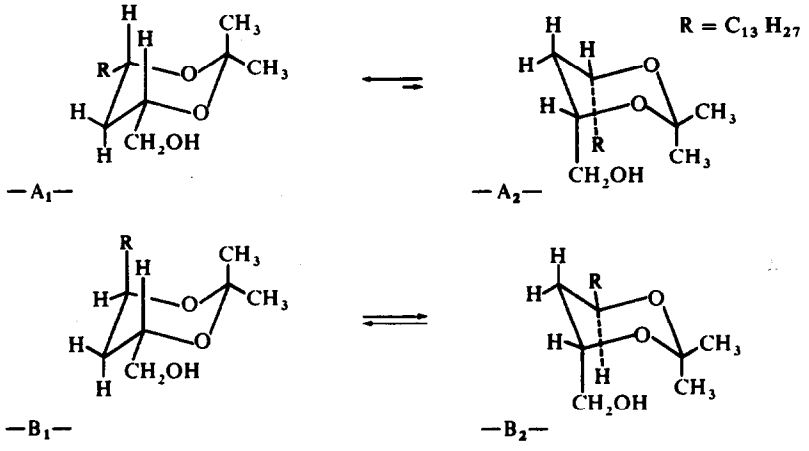


FIG. 1 NMR Spectrum of Compound IVa in Pyridine

Recently, pyridine-induced solvent shifts in the NMR spectra of hydroxylic compounds were studied.[3] The solvent shifts observed were rationalized in terms of specific solute-solvent complexes between the pyridine molecule and the polar OH function in the solute molecule. It has been reported that in saturated cyclic systems, protons occupying positions 1,3 diaxial, vicinal or geminal to a OH function are deshielded. This seemed to be a suitable method for distinguishing between C-2 and C-4 protons. Indeed when the NMR spectrum of compound IVa was taken in pyridin, the expected change in the magnetic anisotropy resulted. For the first time, the C-2 proton appeared separately from the C-4 one. (Fig. 1).

The 4,6-disubstituted 1,3-dioxocyclohexanoic compound, having two asymmetric centers can appear in the four following structures:



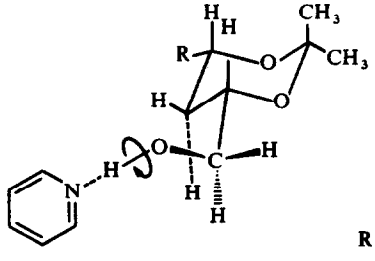
Among these structures, the A_2 conformer seems unlikely due to the three 1,3-diaxial interactions, so that only three possibilities remain.

Irradiation at δ 3·84, the center of the C-1 protons signal changed the C-2 pattern to a double doublet ($J = 3{\cdot}6$; $J = 11{\cdot}9$ c/s). (Fig. 1, c). The coupling constant of 11·9 c/s is characteristic for 1,2-*trans* diaxial relationship and clearly indicates that the C-2-H is axial. Irradiation of the C-2-H at δ 4·18 changed the $H_1H_{1'}$ pattern as expected to an AB system δ 3·80d and δ 3·88d. The conclusion that C-2-H is axial eliminates the possibility of compound IV existing in the B_2 conformation. Although the C-4 proton is not clearly seen, it seemed, even at that stage, that the most probable structure of the acetonide is A_1. Had B been the structure, it would have occurred in each of the two possible conformers B_1 and B_2, and such a situation would have given rise to different J-values in the NMR spectrum.

To support this conclusion, the cyclic sulphite was synthesized using $SOCl_2$. It is known from literature,[4] that there is a difference of 0·5–1·3 ppm between the chemical shifts of an axial and an equatorial proton when found in a 1,3 position to a sulfite. Thus, even if the fine structure of the C-2-H and C-4-H could not be observed in the NMR spectrum, their chemical shifts should be significant. The sulfite can exist theoretically in two conformers, in which the S=O group is either axial or equatorial. When compound II was treated with $SOCl_2$ in pyridine, the two isomeric sulfites were

obtained; in the IR spectrum two peaks at 1190 and 1240 cm^{-1} were observed and in the NMR two multiplets appeared at δ 5·02 and at δ 4·61. Heating the mixture of the two sulfites, resulted in the more stable axial isomer.[5] This isomer could also be obtained when compound II was treated with $SOCl_2$ in CH_2Cl_2 in presence of $CaCO_3$. In the IR spectrum of the resulting product (V) only the peak at 1190 cm^{-1} attributed to the axial isomer appeared. In the NMR spectrum of this axial sulfite (V) only one multiplet appeared at δ 5·02 for the C-2-H and C-4-H, separately from the C-1 protons which appeared at δ 4·14 m, proving that these protons are both in the same axial or equatorial configuration. It seems more probable that the two protons are axial, since if both of them were equatorial, the resulting structure would be very unstable owing to 1,3 interactions. The last evidence together with the NMR data from the hydroxy acetonide IVa taken in pyridine indicate clearly that the structure of the acetonide (IVa) is A_1 and that this holds true also for the sulfite (V). The two OH groups attached to C-2 and to C-4 must therefore be in the same absolute configuration. This is in good agreement with the small $[\alpha]_D$ values found for other closely related compounds isolated from avocado.

Of interest in this connection, was the spectra of the two C-3 protons of the acetonide (IVa) taken in different solvents. In the NMR spectrum taken in $CDCl_3$, the C-3 protons appeared as a multiplet at δ 1·32 ppm, while in pyridine they appeared as two multiplets at δ 1·30 and at δ 1·62. Irradiation of the signal at δ 1·62 changed the multiplet of the C-2 proton into a signal which looks like a double triplet (J = 11·9; J = 6 c/s) (Fig. 1, b) cancelling the 3·6 c/s coupling. This proves that, of the protons linked to C-3, the irradiated one was the equatorial. The shift of about −0·3 ppm, observed for the equatorial C-3 proton which resulted from change of solvents, is close to the value of −0·27 ppm reported for 1,3 diaxial deshielding by the solute solvent complex. A similar steric relationship between the equatorial C-3 proton and the OH group on C-1 means that the OH preferably orients itself so that the C—O bond is approximately parallel to the equatorial C-3-H. The same conclusion was derived from the comparison of the chemical shift of C-2 proton in $CDCl_3$ (δ 4·0 ppm) with the value in pyridine (δ 4·2 ppm). According to Demarco's values,[3] such a solvent shift (−0·2 ppm) shows that the OH orients itself so that the angle with C-2-H is approximately +60°, i.e. parallel to the equatorial C-3 proton, (it is clear that 60° to the other direction is unreasonable). In this way the pyridine ring prefers the least hindered position:

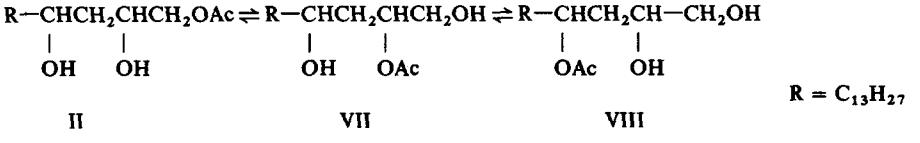


R = $C_{13}H_{27}$

During the synthesis of the acetonide (IVb), its great sensitivity to acid was observed. It could only be obtained in a pure state by the use of a mild Lewis acid like anhydrous $CuSO_4$. When compound II was treated with a stronger acid, or even when its solution

in ethanol was kept for a few days at room temperature, it gave rise to two new compounds; on TLC, additional two spots were seen. One of them, the slower moving spot (VIII) was obtained in a pure state by column chromatopaphy, but the other compound (VII) could not be obtained even by preparative TLC; it immediately isomerized to compounds II and VIII. All these three compounds either separately or in various mixtures yielded, upon acetylation, only one compound—the triacetate which we reported before (part I). This proves that the only difference between these three compounds is in the location of the acetate group.

In the NMR spectrum of compound VIII the 8-line pattern attributed to the C-1 protons appears at δ 3·50 (2H) clearly indicating that C-1 bears a free hydroxyl group (Table 1 part I).[1] The multiplet which appears at δ 3·75 (1H) must be attributed to the C-2 proton, as irradiation at this point changed the C-1 pattern into an AB system. The fourth low field proton which appears at δ 4·94 quin (J = 6·5) (1H) clearly shows that the acetate group is on C-4, and indeed irradiation at this point had no effect on the C-1 pattern. These observations ascertain that compound VIII is the 1,2-dihydroxy-4-acetoxy analogue of compound II. The third compound (VII), which could not be obtained in a clean state is most probably the C-2 acetoxy isomer which enables the transesterification from II to VIII to occur



On TLC of the crude petrol ether extract of avocado seed, three spots with R_f values equal to those of compounds II, VII and VIII were observed. These three spots were also seen in the TLC plates of the crude extracts after its separation to olefinic and acetylenic fractions. From these fractions, compounds IX and X, the acetylenic and olefinic analogues of compound VIII were separated using preparative TLC.

The NMR spectra of these compounds showed for the oxygenated moiety exactly the same pattern as that of compound VIII. Additional peaks at δ 1·90t (J = 2·5) (1H) and δ 2·10m (2H) typical for acetylenic protons, were observed in the spectrum of compound IX, and at δ 5·80m (1H) and δ 4·94m (2H) typical signals for the vinilic protons in the case of compound X.

The acetylenic and olefinic analogues of compound VII could not be separated just as was the case with compound VII itself.

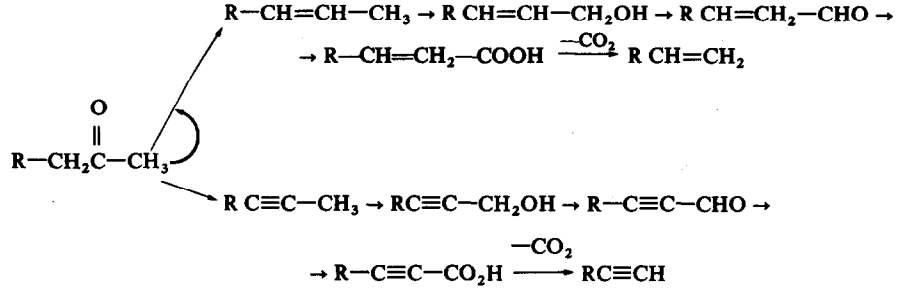
We can now account for six pairs of natural compounds isolated from avocado. In part I we reported on the trihydroxy, the 1-acetoxy, the 4-keto and the furan derivatives of the n-C_{17}-ω-unsaturated compounds. The results described in this paper prove the existance of two more pairs—the 2-acetoxy and the 4-acetoxy derivatives. These six pairs have each the same structure but for the unsaturated end.

Another natural product possibly present in the petrol ether extract seems to be the diacetate derivative of compound II. This compound appeared as a contamination of the 1-acetoxy and the 4-keto derivatives. Its presence was indicated in the mass spectra of these compounds where a molecular peak at m/e 356 appeared.

Other non polar compounds like triglycerides and alkans which were isolated from

the crude extract and correspond to the highest spot on TLC of this extract need further investigation.

When considering the possible biosynthesis of the C_{17} group of compounds we reported, it seems logical that they originate from a C_{18} precursor. The steps involved could be:



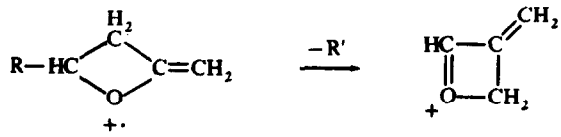
In search for such precursors we succeeded, up to now, to isolate a mixture of the two C_{18} alcohols. In the mass spectrum a pair of peaks at *m/e* 314 and *m/e* 316 appeared. Other peaks which appear as pairs at *m/e* 296, 298; 283, 285; 265, 267; 247, 249; 239, 241 etc., together with the peaks at 105; 87; 69 indicate clearly that the oxygenated site of these compounds is the same as that of the previously described C_{17}-trihydroxy compounds.

These and other compounds found in avocado are now being further investigated.

Mass spectrometry. The mass spectrum of compound IVb is significant for acetonides.[6] It shows a very weak molecular peak at *m/e* 370 (0·2%), (the base peak being *m/e* 43). The molecule loosing a Me group gives rise to an extremely stabilized carbonium ion. This ion is responsible for the relatively intensive peak at *m/e* 355 (25%). Fragmentation of compound IVb is shown in Scheme 1. Support to this scheme is obtained by comparison of the fragments of the acetonide (IVb) with those of its hexadeuterio analogue, and by the appearance of several metastable peaks. In the lower mass range two of the most prominent peaks at *m/e* 43 (100%) and *m/e* 59 (34%) shift in the mass spectrum of the hexadeuterio derivative to *m/e* 46 and *m/e* 65 respectively, as is well known for acetonides.[6] In the mass spectrum of the hydroxy acetonide (IVa) two intensive peaks appear at *m/e* 313 (33%) (M—CH_3) and *m/e* 297 (25%) (M—CH_2OH), owing to the α-cleavage. (The base peak being *m/e* 59 $(CH_3)_2\ C = \overset{+}{O}H$). Further fragmentations of these peaks are shown in Scheme 2. No molecular peak for compound IVa could be observed—this too is a well known phenomenon in acetonides.

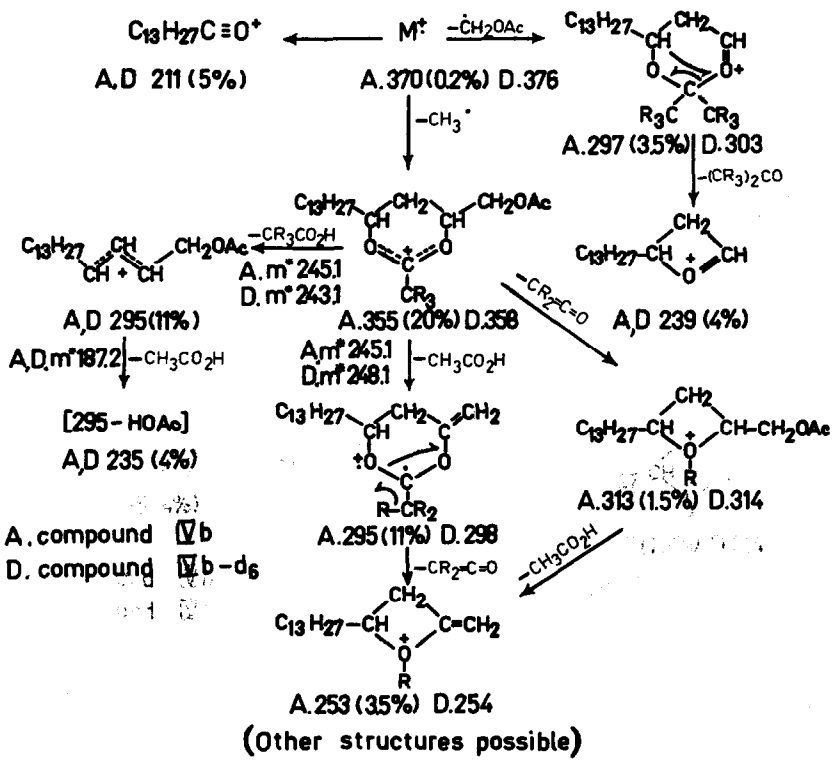
The mass spectrum of the axial sulfite (V) shows a fragmentation which is typical for cyclic sulphites[7] as shown in Scheme 3. No distinction could be seen between this mass spectrum and that of the equatorial isomer (VI).

In the spectrum of the carbonate (III) apart from the weak molecular peak at *m/e* 356 (0·4%) only two other significant peaks in the high mass range could be observed: *m/e* 252 (17%) $[M—CH_3CO_2H—CO_2]^+$ and *m/e* 235 (27%) $[M—CH_3CO_2H—CHO_3^{\cdot}]^+$ which may be the same fragments as that derived from the acetonides. In the low mass range apart from the intense peaks at *m/e* 44 (100%) and *m/e* 43 (98%) a quite intense peak appeared at *m/e* 69 which can be rationalized in the following manner:

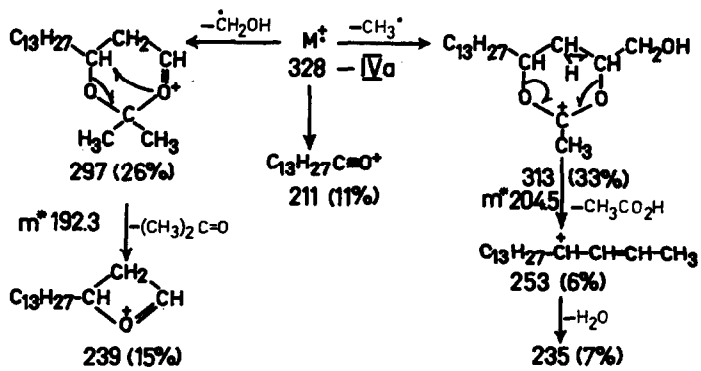


m/e 252 *m/e* 69 R = $C_{13}H_{27}$

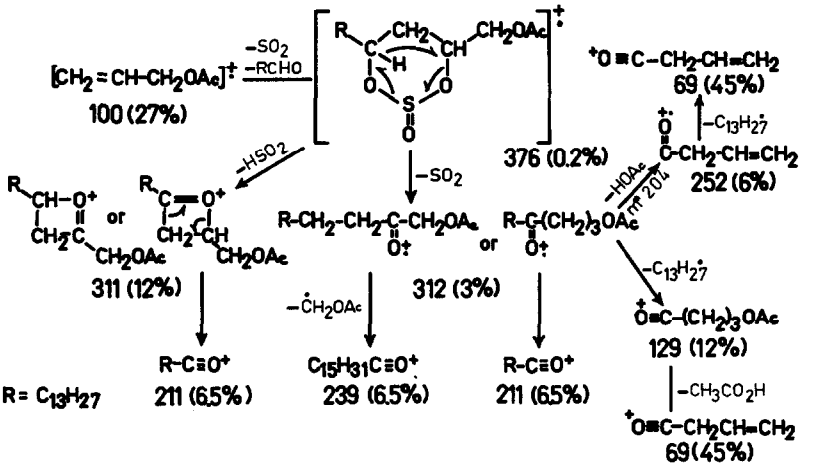
SCHEME 1



SCHEME 2



SCHEME 3



EXPERIMENTAL

For experimental conditions see part I.[1]

Preparation of the carbonate (III) *from* II. A 10% soln of $COCl_2$ in benzene was slowly added to a soln of II (100 mg) in dry benzene (5 ml) and dry pyridin (3 ml), cooled in an ice bath. The mixture was then kept overnight at room temp. After destroying the excess of $COCl_2$ by slowly adding ice water, the residue was taken into ether, washed with dil HCl, water, $NaHCO_3$ soln and again with water. The crude product obtained after evaporation of the dried soln, was filtered through a silicagel column and then crystallized from hexane, m.p. 87°. $[\alpha]_D^{23}$ +40° (*c*, 0·5 $CHCl_3$), $\nu_{max}^{CHCl_3}$ 1735, 1230, 1120, 1030 cm^{-1}. (Found: C, 67·18; H, 10·12. $C_{20}H_{36}O_5$ requires: C, 67·38; H, 10·18%).

Preparation of the acetonide (IVb) *from* II was described in part I.[1]

Hydrolysis of compound IVb *to compound* IVa. Compound IVb (100 mg) was dissolved in a 1%-methanolic KOH soln and kept at room temp for several hrs. When the hydrolysis was completed (TLC) the soln was neutralized with dil ACOH, evaporated to a small volume and taken into ether. The ether soln was washed with water, dryed and evaporated. The compound crystallized from hexane m.p. 38–40°, $\nu_{max}^{CHCl_3}$ 3450, 1190, 1160 and 1120 cm^{-1}. (Found: C, 72·95; H, 12·40. $C_{20}H_{40}O_3$ requires: C, 73·12; H, 12·27%).

Preparation of the sulfites V *and* VI *from* II. (a) To a cool soln of II (200 mg) in dry pyridine (3 ml) $SOCl_2$ (0·5 ml) was added. The soln was allowed to come to room temp in about 1 hr and then the excess pyridine and $SOCl_2$ were removed under low press (0·5 mm Hg). The residue was taken into $CHCl_3$, washed with cold dil HCl, $NaHCO_3$ and water. The product after evaporation was submitted to chromatography on neutral deactivated alumina column. Elution with petrol–ether : chloroform 15 : 1 gave the two isomers, first the axial sulphite and then the equatorial one. Compound V m.p. 35° (hexane), ν_{max}^{KBr} 1730, 1240, 1190 cm^{-1}. (Found: M^+ 376; C, 60·51; H, 9·68; S, 8·43. $C_{19}H_{36}O_5S$ requires: MW 376. C, 60·61; H, 9·64; S, 8·5%). Compound VI m.p. 56° (hexane), ν_{max}^{KBr} 1730, 1240 cm^{-1}. (Found: C, 60·53; H, 9·70; S, 8·40. $C_{19}H_{36}O_5S$ requires: C, 60·61; H, 9·64; S, 8·50%).

(b) Compound II (100 mg), in dry CH_2Cl_2 (10 ml) containing $CaCO_3$ (100 mg), was treated with $SOCl_2$ (0·25 ml). After stirring for 1 hr at room temp, the soln was filtered then washed with ice water, $NaHCO_3$ and water again. The product obtained, following evaporation, was filtered through neutral alumina to give the axial sulfite V.

Isomerization of VI *to* V. A mixture of V and VI was heated for 10 min at 200°. The crude cooled material showed in the NMR spectrum the disappearance of compound VI. After chromatography on neutral alumina, only compound V was obtained.

Isomerization of compound II *to* VII *and* VIII. Compound II (100 mg) was dissolved in ether (10 ml) containing catalytic amounts of *p*-TsOH, and the soln kept overnight at room temp. It was then neutralized with $NaHCO_3$, washed twice with water, dryed and evaporated under reduced press. The residue which

gave on TLC three spots was chromatographed on a column of neutral alumina. Compound II was the first to be eluted. From the following eluate compound VIII was separated by preparative TLC. It crystallized from hexane as low m.p. crystals, $\nu_{max}^{CHCl_3}$ 1735, 1250, 1110, 1065, 1030 cm^{-1}. (Found: M^+ 330 $C_{19}H_{38}O_4$ requires: MW 330).

Acetylation of II *or* VIII. Compound II or VIII (50 mg) was dissolved in pyridine 0·5 ml) and Ac_2O (0·5 ml), and was kept overnight at room temp. After the usual workup, the oily triacetate described in part I resulted.

Isolation of compounds IX *and* X from the crude extract was carried out in the procedure described for the preparation of VIII. The olefinic compound X was obtained as an oily substance. Compound IX was crystallized from hexan m.p. 56–57°, ν_{max}^{KBr} 3500, 3400, 3260, 2120, 1725, 1250, 1120, 1100, 1090, 1050, 1035, 1020 cm^{-1}. (Found: M^+ 326 $C_{19}H_{34}O_4$ requires: MW 326).

Acknowledgement—The authors are grateful to Mrs. R. Melamed for enthusiastic help in carrying out the experiments.

REFERENCES

[1] Y. Kashman, I. Néeman and A. Lifshitz, *Tetrahedron* **25**, 4617 (1969).
[2] L. M. Jackman, *Application of NMR spectroscopy in organic chemistry* p. 55. Pergamon Press, New York (1966).
[3] P. V. Demarco, E. Farkas, D. Doddrell, B. L. Mylari and E. Wenkert, *J. Am. Chem. Soc.* **90**, 5480 (1963).
[4] [a] H. F. Van Woerden and E. Havinga, *Rec. Trav. Chim.* **86**, 341 (1967), and refs cited therein;
[b] S. Sarel and V. Usieli, *Israel J. Chem.* **6**, 885 (1968).
[5] P. C. Lauterbur, J. G. Pritchard and R. L. Vollmer, *J. Chem. Soc.* 5307 (1968).
[6] H. Budzikiewicz, C. Djerassi and D. H. Williams, *Mass spectrometry of organic compounds* p. 476. Holden-Day (1967).
[7] *Ibid.* p. 498.