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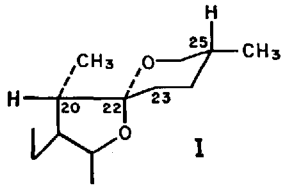
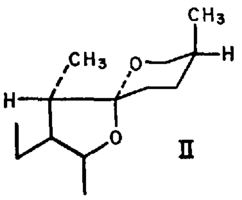
Steroidal Sapogenins. XXX Stereochemistry of the Side Chain

A number of workers have recently investigated the stereochemistry of the stereoidal sapogenin side chain[1]. The fact that naturally occurring sapogenins have isomerism at C_{25} was established by SCHEER, KOSTIC, and MOSETTIG, and by JAMES[2]. Work at this laboratory[3] has shown that natural sapogenins have the 20α configuration whereas the unnatural 20-isosapogenins (also called ana-[4], cyclopseudo-[5], and neosapogenins[6]) have the 20β orientation. It has also been recently demonstrated that a true equilibrium is established as a result of heating sapogenins with alcoholic HCl[7], sarsasapogenin and smilagenin each giving a mixture containing approximately 20% of the former and 80% of the latter.



I



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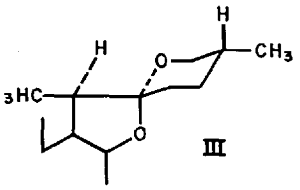

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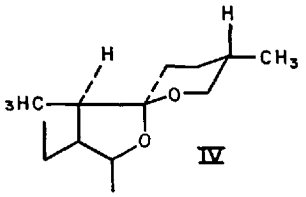

IV

From the foregoing considerations (presented in greater detail in references[8]) the side chain formulation of smilagenin and related 20α, 25D-sapogenins is best represented by I.

Recently, we prepared a number of 20β, 25D- and 20β, 25L-sapogenins[1]. From a comparison of the specific rotations of these sapogenins with their 20α analogues (Table), the author has deduced that formulation II best represents sarsasapogenin and 20α, 25L-sapogenins; formulation III is given to 20-isosmilagenin and related 20β, 25D-sapogenins; and formulation IV best fits 20-isosarsasapogenin and related 20β, 25L-sapogenins.

The basis for the above assignments is the assumption that the highly polar asymmetric center at C_{22} is responsible for the major portion of the observed $[\alpha]_D$ of sapogenins and that the centers at C_{20} and C_{25} have only a minor effect on $[\alpha]_D$. It follows that any major change in the $[\alpha]_D$ of sapogenins must be ascribed to a change at C_{22}.

Let us examine the data which substantiates this assumption. Columns 1 and 2 of Table give the $[\alpha]_D$ of a number of 20α and 20β pairs known to differ at C_{25} in each series[2]. The $[M]_D$ differences for the 20α series are shown in column 3 and are of obvious low magnitude. Column 4 gives the same data for the 20β series and shows a pronounced dextrorotatory shift of large magnitude. We have shown[3] that the C_{25} configurations of sapogenins of the 20β series are identical to their corresponding 20α analogues. Accordingly, we can rule out C_{25} isomerism as a factor in the pronounced dextrorotatory shift observed in the 20β series since we have demonstrated that corresponding C_{25} differences in the 20α series have little effect on rotation. Similarly we can demonstrate that the C_{20} center exerts only a minor effect. Column 6 shows that a change from 20α to 20β in the 25D series has an average effect of about + 45 units. Column 5 shows that the same change from 20α to 20β in the 25L series is of much greater magnitude and of the same order found in column 4 which compares 25L and 25D isomers with the same $C_{20}\beta$ configuration.

One must conclude that the great dextrorotatory shift found in passing to the 20β, 25L series is due to the fact that this group *differs at* C_{22} from its 20β, 25D; 20α, 25D; and 20α, 25L isomers and further that all the other series are *identical at* C_{22}. Furthermore the data supports the view that the highly polar spiroketal C_{22} group is responsible for the major part of the $[\alpha]_D$ values observed with sapogenins. Additional evidence for this view is the fact that whenever the polar spiroketal ring is opened as in the formation of pseudo-, dihydro-, and dihydropseudosapogenins, there is again a pronounced dextro-

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	$[\alpha]_D^0$ *		$[M_D]$** Differences			
Compound	*1* 20α	*2* 20β	*3* ΔE_1[a]	*4* ΔE_2[b]	*5* ΔE_3[c]	*6* ΔE_4[d]
Sarsasapogenin 25L	− 75	+ 31[1]	−17	+374	+440	
Smilagenin 25D	− 71	− 59[1]				+ 50
Markogenin 25L	− 70	+ 13[2]	+17	. . .	+358	
Samogenin 25D	− 74	. . .				. . .
Yamogenin 25L	−129	− 15[2]	0	+364	+473	
Diosgenin 25D	−129	−103[2] −122[3]				+108 + 29
3-Desoxysarsasapogenin 25L	− 73	+ 37[2]	− 8	+400	+444	
3-Desoxysmilagenin 25D	− 71	− 63[2]				+ 32
3-Desoxytigogenin 25D	− 69	− 59[2]				+ 40
Tigogenin 25D	− 67	− 67[2]				0

* $[\alpha]_D$ of 20α series determined in chloroform, 20β series in dioxane and converted to chloroform basis by adding (−5) to each observed dioxane value.

** $M_D = [\alpha]_D \times$ molecular weight/100

[a] $\Delta E_1 = M_D\,[(20\alpha, 25L)\text{-}(20\alpha, 25D)]$

[b] $\Delta E_2 = M_D\,[(20\beta, 25L)\text{-}(20\beta, 25D)]$

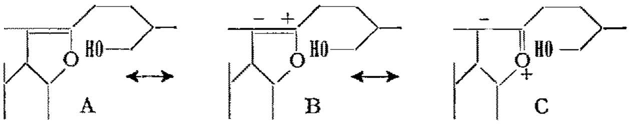
[c] $\Delta E_3 = M_D\,[(20\beta, 25L)\text{-}(20\alpha, 25L)]$

[d] $\Delta E_4 = M_D\,[(20\beta, 25D)\text{-}(20\alpha, 25D)]$

rotatory change in the rotation and the observed $[\alpha]_D$ values are generally near zero[1]. Formulation I has been assigned to smilagenin by several research groups[4] and certainly seems reasonable on the basis of information at hand. It follows from the optical rotation data previously cited that sarsasapogenin is II differing from I only at C_{25}; 20-isosmilagenin is III, differing from I only at C_{20}, and 20-isosarsasapogenin is IV differing from I at C_{20}, C_{22}, and C_{25}. The author proposes that sapogenins of groups I, II, III, IV be called respectively 20α, *22a*, 25D-; 20α, *22a*, 25L-; *20β*, *22a*, 25D-; and *20β*, *22b*, 25L-sapogenins (G. MUELLER and B. RIEGEL first proposed this nomenclature system).

Finally, let us examine the manner in which the series I–IV could be formed by cyclization of the pseudosapogenins. It is probable that pseudosapogenins exist as resonance stabilized hybrids. In the presence of H+, cyclization takes place in the sequence:

$$20\beta \underset{2}{\overset{1}{\rightleftarrows}} \text{Pseudo} \xrightarrow{3} 20\alpha^{5}.$$



Assuming cyclization of the planar forms *B* or *C* of pseudosapogenins, there is no longer need to be bound rigidly by the concept of trans ring closures[6]. The nature of the various isomers which are formed seems to depend entirely on *steric effects*.

Considering first the 20α series, it will be noted that models with C_{22} configuration opposite that of I and II indicate that there would be a strong interaction between the C_{21} methyl and the C_{23} methylene groups. Models show much less interaction between the C_{21} methyl and the smaller oxygen atom as shown in I and II so that these forms have less overall energy and are favored. In the case of II, the interaction of the axial C_{27} methyl with a single hydrogen atom has less effect on the overall energy of the molecule than the C_{21}–C_{23} interactions discussed above.

A different situation occurs in the 20β series. In this case the C_{21} methyl is replaced by a much smaller hydrogen atom, and models show no interaction in either of the two C_{22} possibilities. Under these circumstances steric effects at C_{25} might well determine the direction of ring closure so that in each case the more stable equatorial C_{27} methyl is formed. This would require a *cis* closure in the case of III and a *trans* closure in the case of IV. The foregoing rationalization of the cyclization of pseudosapogenins to give the series I–IV is thus in complete accord with the formulations deduced from the optical rotations of these compounds.

There remains for discussion the infrared spectra of I–IV. Originally, we concluded in agreement with R. N. JONES that the large differences between the infrared spectra of I and II were due to differences in C_{22} configuration[1]. The present evidence renders the above hypothesis untenable. Instead the infrared differences between I and II must be ascribed to the equatorial and axial C_{27} methyl group. The markedly different spectre of III[2]. must be ascribed to the strain produced by the 20β configuration, and that of IV both to the 20β and to the difference in C_{22} configuration.

M. E. WALL

Eastern Regional Research Laboratory, Eastern Utilization Research Branch, Agricultural Research Service, United States Department of Agriculture Philadelphia 18, Pennsylvania, June 11, 1955.

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Zusammenfassung

Die optischen Drehungen einiger 20α, 25D; 20α, 25L; 20β, 25D; und 20β, 25-L-Sapogenine wurden bestimmt. Die ersten drei Serien gaben übereinstimmend linksdrehende Werte, aber die letztere Gruppe erwies sich als rechtsdrehend.

Die Struktur der vier Serien der Sapogenine folgte aus der Analyse dieser Befunde. Der mögliche Mechanismus bei der Entstehung dieser Verbindungen aus Pseudosapogeninen wurde besprochen. Die Autoren gelangen zum Schluss, dass sterische Faktoren an C_{20} und C_{25} die Richtung der Ringschliessung beeinflussen.

Electrolytic Behaviour of Some Carotenoids in Strongly Acid Media

Some time ago, in the course of quite a different investigation[1], we dissolved carotene in isobutylalcohol which was saturated with a solution of 25 % sulfuric acid in water, and we electrolysed this solution in an inverted V-tube[2] between two beakers containing 25 % sulfuric acid in water into which anode and cathode were immersed. A bluish-green compound migrated towards the cathode and accumulated at the cathodic end of the isobutylalcohol layer. L. CHOLNOKY, to whom we are indebted for the gift of carotene and for discussions told us, on inspection of the experiment, that his carotene probably contained epoxides and he thought their salts were involved in the electrolytic migration. As a matter of fact, the yellow carotene itself did not migrate.

To identify the green, ionized compound, we prepared authentic β-carotene epoxides with perphthalic acid following KARRER's[3] method and resolved the resulting mixture by chromatography on a $Ca/OH/_2$ column with petrol-ether. Eight different zones were isolated and zones 6. and 7. were identified as KARRER's luteochrome and β-carotene-diepoxide respectively. The maxima of their absorption spectra in carbon disulfide were: 454 and 480 mμ for luteochrome (KARRER's values: 451 and 482 mμ) respectively 470 and 502 mμ for β-carotene-diepoxide (KARRER's values: 470 and 502 mμ). Both compounds were dissolved in the acidified isobutyl alcohol mentioned above. The initially yellow colour of the solutions turned gradually into blueish-green, as described by KARRER, in chloroform containing hydrochloride acid. These green compounds migrated towards the cathode in the above-mentioned inverted V-tube apparatus, applying 90 V to the 3 cm portion of the tube (internal diameter 2 mm) which was filled with the isobutyl alcoholic solution. The current was 5 mA. The green compound did not pass into the aqueous sulfuric acid but accumulated at the cathodic end of the alcoholic phase; it followed the current after reversal of the electrodes.

Other chromatographic fractions of the oxidized carotene behaved similarly, although their colour, which developed after a time in the acidified alcohol, varied between lavender-blue, violet and green. The coloured compound in some cases weakly passed the phase border into the aqueous acid at the cathodic side.

The cathionic behaviour of these carotenoids may be due either to their loosely bound π-electrons or to the formation of oxoniumsalts on the ring-oxygen atoms.

To test these hypotheses, we performed some further experiments. First we tried to electrolyse the chloroform solution of the blue product from carotene and $SbCl_5$ of the Carr-Price reaction, but failed to detect any migration of the blue boundarise between coloured and uncoloured chloroform. This colour reaction is given by carotenoids irrespective of whether they are pure hydrocarbons or contain oxygen. They are held to be dative compounds of the electron-donor carotenoids and the acceptor Lewis-acids ($SbCl_5$, $SbCl_3$, $AlCl_3$, BF_3, etc). In our case the dative compound evidently did not dissociate into positive and negative ions in chloroform solution.

Then we investigated the deep blue compounds which are formed from carotenoids and strong acids. It is well known that the hydrocarbon carotenoids yield these blue compounds with concentrated sulfuric acid, while carotenoids containing oxygen, e.g. violaxanthine, react even with dilute hydrochloric acid. Not much seems to be known, however, about these compounds.

We separated α- and β-carotene chromatographically from each other and examined their reaction with sulfuric acid of varying concentration. They dissolve in concentrated acid to a beautiful peacock-blue solution which however fades through ultramarin to an unsightly violet. Using sulfuric acid diluted with increasing amounts of water, we found that the peacock-blue colour is relatively stable at and beyond 15 % water content and the blue colour develops up to about 28 % water contents. Using a solution of carotene in benzene and shaking it with sulfuric acid of different concentration, we found that a partition equilibrium is established between the acid and benzene phase. At about 84 % H_2SO_4 and above all carotene passes into the acid, while below 72 % all of it remains in the benzene or reverts into benzene solution if a stronger acid is diluted with water. At concentrations between 72 and 84 % H_2SO_4, the carotene is distributed between the yellow benzene and the blue acid phase reversibly. It should be proved, however, whether the reverted yellow pigment is genuine carotene or a derivate therefrom. We also wish to investigate the partition equilibrium between these phases quantitatively. It is interesting to note that the upper and lower concentration limits of the sulfuric acid at both sides of this partition equilibrium approximate very closely to the composition for $H_2SO_4 \cdot H_2O$ and $H_2SO_4 \cdot 2H_2O$ respectively, although in view of the fact that carotenoids containing oxygen sometimes give the blue colour even with dilute acids, this agreement may prove to be a mere coincidence.

These blue solutions were subjected to an electric field strength of about 1 V/cm in the inverted V-tube apparatus. To stabilize the boundary between coloured solution and the underlying strong sulfuric acid, the former was slightly diluted with water or methanol. To our great surprise these blue derivates of the carotenes migrated to the anode. Their ionic mobilities were of the order of 10^{-4}, which may be regarded as normal in viscous sulfuric acid media. The fractions of the chromatogramm of oxydised carotene mentioned above were treated similarly and this time they too all migrated towards the anode! The cationic or anionic behaviour thus depends upon whether we work in aqueous acid solutions or in acidified isobutyl alcohol (or amyl alcohol). The 6th fraction (luteochrome) dissolved to a grayish-green colour, the 7th fraction (diepoxide) to greenish-blue while the others varied between brown and violet.

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