[CONTRIBUTION FROM THE EASTERN REGIONAL RESEARCH LABORATORY[1]]

Steroidal Sapogenins. XLV. Effect of Side Chain Isomerism on Rate of Conversion to Pseudosapogenins[2]

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A study of the pseudomerization rates of 20α,25D-, 20α,25L-, 20β,25D- and 20β,25L-sapogenins shows that in the 20α-series the 25L-sapogenins are pseudomerized much more rapidly than the corresponding 25D-compound; the reverse is true in the 20β-series. The application of these findings to the question of the stereochemistry of the spiroketal side chain is discussed in detail.

The conversion of sapogenins to the corresponding pseudosapogenin diacetates by means of acetic anhydride at elevated temperatures (pseudomerization) is a key reaction in hormone technology. The reaction was first discovered by Marker and coworkers,[3a,b,c] the standard operating procedure requiring heating the sapogenin with acetic anhydride in sealed vessels at 200° for approximately 10 hr. More recently aluminum chloride[4] or pyridine hydrochloride[5] has been used in refluxing acetic anhydride to convert sapogenins to pseudosapogenins. Cameron and his associates[6] in an excellent study of the pseudomerization reaction recommended the use of octanoic anhydride or octanoic acid at rather elevated temperatures. None of the above references indicated that the effect of side chain isomerism on the pseudomerization rate had ever been studied.

In continuation of our previous studies on the configuration of the spiroketal side chain[2,7] we have studied the pseudomerization of natural sapogenins of the 25D- and 25L-series and of their analogous isomers in the 20β-series[7] and find that differences in side chain structure can greatly change the rate of this reaction.

The initial experiments were conducted with natural sapogenins of the 25D- and 25L-series. Exploratory work indicated that under the conditions recommended by previous workers[3-6] all the sapogenins were converted rapidly to pseudosapogenins plus other products and rate differences could not be ascertained. The method finally adopted utilized acetic anhydride containing a trace of acetic acid and a reaction temperature of 170°. Conversion to pseudosapogenins was followed by infrared measurements, paper chromatography and most conveniently by ultraviolet measurements at 215 mμ. The results are shown in Table I.

From the data it is apparent that the 25L-sapogenins sarsasapogenin, yamogenin, and neotigogenin were pseudomerized 2–3 times more rapidly than their corresponding 25D-analogs, smilagenin, diosgenin and tigogenin, respectively. The data thus indicate that the 25L-spiroketal side chain is less stable than the corresponding 25D-series in complete agreement with earlier experiments which demonstrated that the 25L-series was labile toward hydrochloric acid in contrast to the more stable 25D-series.[3b,7b,8] On the basis of conformational analysis,[8,9] molecular rotation deductions,[7d] and experiments with isomeric 25D- and 25L-sapogenins which demonstrated that identical compounds were obtained after destroying the asymmetric center at C_{25},[10] we conclude that in natural sapogenins the spiroketal side chain isomers have the structure 20α,22a,25D-(Ia) and 20α,22a,25L-spirostane (Ib).

TABLE I

PSEUDOMERIZATION OF NATURAL SAPOGENINS IN ACETIC ANHYDRIDE AT 170°

Time, hr.	Conversion, %, to pseudosapogenin from: Sarsasapogenin	Smilagenin	Yamogenin	Diosgenin	Neotigogenin	Tigogenin
0.5	8.0					
1.0	18.5	4.5			39.5	
1.25					50.0[a]	
1.5	39.5					
1.8	50.0[a]					
2.0	58.5	12.0			80.0	
3.0	88.5		51.0[a]	20.0		24.5
4.0	98.5	24.5				
5.0		45.5	72.0	41.0	100.0	29.5
5.6		50.0[a]				
6.0				50.0[a]		
7.0		60.5	92.0	63.5		38.0
8.6						50.0[a]
11.0		80.5		74.0		67.5
17.0		92.0	99.0	92.0		86.0
21.0		99.0		98.5		99.0

[a] All 50% conversion times were calculated except for yamogenin.

Another factor which affects the rate of pseudomerization is the configuration at C_5. Thus the data in Table I show that sarsasapogenin and neotigogenin (C_5-*cis* and *trans*, respectively) are converted to pseudosapogenins more rapidly than yamogenin ($\Delta^{5(6)}$). In the 25D-series, smilagenin

(1) A laboratory of the Eastern Utilization Research and Development Division, Agricultural Research Service, United States Department of Agriculture. Article not copyrighted.

(2) Paper XLIV, M. E. Wall and H. A. Walens, *Chemistry & Industry*, 818 (1957). Presented at the Fall Meeting of the American Chemical Society, New York, N. Y., September 8–13, 1957.

(3) (a) R. E. Marker, THIS JOURNAL, **62**, 3350 (1940); (b) **69**, 2167 (1947); (c) R. E. Marker, E. M. Jones and E. L. Wittbecker, *ibid.*, **64**, 468 (1942).

(4) D. H. Gould, H. Staeudle and E. B. Hershberg, *ibid.*, **74**, 3685 (1952).

(5) W. G. Dauben and G. J. Fonken, *ibid.*, **76**, 4618 (1954).

(6) A. F. B. Cameron, R. M. Evans, J. C. Hamlet, J. S. Hunt, P. G. Jones and A. G. Long, *J. Chem. Soc.*, 2807 (1955).

(7) (a) M. E. Wall, S. Serota and C. R. Eddy, THIS JOURNAL, **77**, 1230 (1955); (b) M. E. Wall, S. Serota and L. P. Witnauer, *ibid.*, **77**, 3086 (1955); (c) M. E. Wall and H. Walens, *ibid.*, **77**, 5661 (1955); (d) M. E. Wall, *Experientia*, **11**, 340 (1955).

(8) R. K. Callow and V. H. T. James, *J. Chem. Soc.*, 1671 (1955).

(9) (a) D. A. H. Taylor, *Chemistry & Industry*, 1066 (1954); (b) J. B. Ziegler, W. Rosen and A. C. Shabica, THIS JOURNAL, **77**, 1223 (1955).

(10) (a) I. Scheer, R. B. Kostic and E. Mosettig, *ibid.*, **77**, 641 (1955); (b) R. K. Callow and R. N. Massy-Beresford, *Chemistry & Industry*, 1146 (1956).

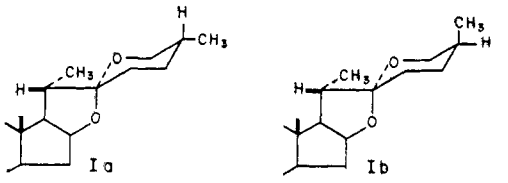

Fig. 1.

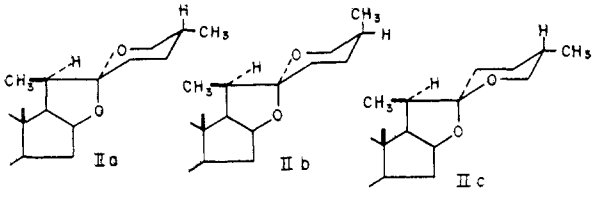

Fig. 2.

(C_5 *cis*) is somewhat more reactive than the C_5 *trans* or $\Delta^{5(6)}$-analogs.[11]

We next studied the pseudomerization rates of the new class of sapogenins formed by treating pseudosapogenins with acetic or dilute hydrochloric acid[7a,c,9b,12] Recently we have demonstrated by an unequivocal partial synthesis[2] that the acetic acid cyclization of 25D-pseudosapogenins gives a product which can be classified as a 20β,22a,25D-spirostane (IIa). The question then arises as to the structure of the corresponding 25L-series. These compounds were prepared in the same manner and have properties quite similar to those in the analogous 25D-series.[7a,c] It seems most likely that both the 25D- and 25L-sapogenins under discussion have the 20β-configuration. Accordingly the 20β,25L-series must be represented by formulations IIb or IIc. As shown in Table II, a study of the pseudo-

TABLE II

PSEUDOMERIZATION OF 20-ISO- AND 20,22-ISOSAPOGENINS

Time, min.	Conversion, %, to pseudosapogenin from— 20,22-Isosarsasapogenin Ac_2O[a]	20,22-Isosarsasapogenin Heat[b]	20-Isosmilagenin Ac_2O[a]	20-Isosmilagenin Heat[b]	20,22-Isoyamogenin, heat[b]	20-Isodiosgenin, heat[b]
15	10	8	60	62	20	50
30	27	10	80	59	23	48
45	49					
60	72		80		26	45
90	88					
120	100	15	80	48		

[a] Temperature 100°. [b] Temperature 187°.

merization rates of 20β,25D- and 20β,25L-sapogenins indicated that the latter group was converted to pseudosapogenin at a rate much slower than the former. This information rules out consideration of formulation IIb and establishes IIc, 20β,22b,-25L-spirostane, as the probable correct structure for the 25L-series.[13] Molecular models of IIa and IIc indicated that the latter might be expected to have the more stable structure.[14]

Comparing formulations Ia, Ib, IIa and IIc, it will be noted that the last has a C_{22} configuration opposite to the first three. We have noted that sapogenins to which we assign structure IIc are markedly more dextrorotatory than the other types.[7a,c] In an earlier paper[7d] we arrived at the formulations Ia, Ib, IIa and IIc from deductions based on molecular rotation differences which we now see to be in complete agreement with deductions based on partial synthesis[2,10b] and stability toward pseudomerization.

Since the structures of the various types of sapogenins now seem to be established with reasonable certainty, we should like to suggest a system for formal and trivial nomenclature exemplified as

Formal name	Trivial name
20α,22β,25D-Spirostan-3β-ol	Smilagenin
20α,22β,25L-Spirostan-3β-ol	Sarsasapogenin
20β,22β,25D-Spirostan-3β-ol	20-Isosmilagenin
20β,22α,25L-Spirostan-3β-ol	20,22-Isosarsasapogenin

The proposed formal nomenclature is essentially a modification of the widely accepted recommendations of the 1950 CIBA conference on steroid nomenclature.[15a,b] The present proposals make provision for isomerism at C_{20} and C_{25} which were not recognized at the time of the 1950 conference. The use of "α" or "β" to designate configuration at C_{20} is based on the relationship to natural sapogenins to bile acids and cholesterol at C_{20} which has been reviewed in an earlier paper.[16] The use of 25D or 25L to denote C_{25}-configuration is based on the work of James who related smilagenin to D-glyceraldehyde.[17] For use of 22α or 22β, *cf.* ref. 15b.

Sapogenins with the 20β-configuration were discovered almost simultaneously in a number of laboratories. The various research groups proposed

(11) The explanation for the above observations is obscure. The results are reminiscent in a reverse sense of the long range effects found by D. H. R. Barton and A. J. Head, *J. Chem. Soc.*, 932 (1956), who found that differences in side chain structure influenced the rate of conversion of 5α,6β-dibromosteroids to the corresponding 5β,6α-analogs. In view of these findings it is not surprising that differences at C_5 may affect side chain reactivity.

(12) R. K. Callow, D. H. W. Dickson, J. Elks, R. M. Evans, V. H. T. James, A. G. Long, J. F. Oughton and J. E. Page, *ibid.*, 1966 (1955).

(13) The structures IIa and IIb are identical except for configuration at C_{25}, the former having an equatorial and the latter an axial methyl group attached to C_{25}. A compound with structure IIb should be more susceptible to pseudomerization than one with structure IIa. Compare, for example, the previously discussed case of spirostanes with structures Ia and Ib which are identical to IIa and IIb except for C_{20}-configuration. It will be recalled that Ib (axial C_{27}-methyl) pseudomerized more rapidly than Ia (equatorial C_{27}-methyl). The observed fact that the 20β,25L-spirostanes were *more stable* than the 20β,25D-spirostanes (IIa) is inconsistent with formulation IIb as compounds with such structure would be expected to be *less stable* than IIa. Since the structure of IIa has been established by an independent line,[2] the 20β,25L-spirostanes must have formulation IIc.

(14) The spirostanes with structures IIa and IIc both have equatorial methyl groups of opposite configuration at C_{25} and differ in configuration at C_{22}. As a result the C_{21}-methyl group interacts with different groups in ring F. In the case of compounds with structure IIa the interaction is with the C_{23}-methylene group (van der Waals radius = 2.0 Å.); with compounds of structure IIc the interaction is with the smaller C_{26}-oxygen atom (van der Waals radius 1.4 Å.). Hence, the greater stability of IIc toward pseudomerization is in agreement with theoretical considerations. From these same considerations formulation IIb should be much less stable than IIc.

(15) (a) R. S. Cahn, *Chemistry & Industry*, SN 1, June 23, 1951; (b) A reviewer has called to our attention a 1955 IUPAC report, *Compt. rend. Dix-huitieme Conf., Zurich*, 20–28 Juillet, 1955. In this report it is recommended that the use of 22a or 22b, recommended in the 1950 conference, ref. 15a, be replaced by the terms α or β applied to the C_{22}–C_{23} bond. At the suggestion of both the reviewer and the Editor, we are adopting this nomenclature.

(16) M. E. Wall, C. R. Eddy and S. Serota, THIS JOURNAL, **76**, 2849 (1954).

(17) V. H. T. James, *Chemistry & Industry*, 1388 (1953).

the terms 20-iso-,[16] neo-,[9b] ana-[18] and cyclopseudo-[9a] to be used as prefixes to the trivial name of the parent sapogenin. As now seems apparent, the use of 20-iso does not cover all cases. With the structure of the 20β-sapogenins now on firm ground there no longer seems to be a need for the non-specific terms neo, ana, or cyclopseudo. We therefore propose 20-iso or 20,22-iso as shown above.

Experimental

Pseudomerization of Sapogenins. (a) Micro Procedure.—To a Pyrex tube (8 mm. × 12.5 cm.) sealed at one end was added 0.2 g. of sapogenin acetate followed by 0.5 ml. of acetic anhydride containing a trace of acetic acid.[19] The tube was sealed close to the open end, wrapped in a copper spiral,[20] immersed in a heating bath and removed after the desired time interval. The pseudomerization runs were conducted at temperatures of 100, 170, 180 and 188° using heating-baths of water, butyl Cellosolve, aniline and propylene glycol, respectively. Trial runs quickly indicated that for the acetic anhydride pseudomerization of natural sapogenins, a temperature of 170° was best for determination of pseudomerization rates, the reaction proceeding too slowly at lower and too rapidly at higher temperatures. For 20-iso- or 20,22-isosapogenins, a temperature of 100° was found most suitable. In experiments in which these last named sapogenins were heated without addition of acetic anhydride, a temperature of 188° was convenient with quantities of 0.10 g.

Determination of Pseudosapogenins.—After the appropriate heating periods, the tubes were opened, and the contents refluxed one-half hour with 20 ml. of methanol containing 1.0 g. of potassium hydroxide. Three volumes of water was added, and the precipitated product filtered, thoroughly washed with hot water and dried *in vacuo* at 80°. The ultraviolet absorption of a weighed sample (0.025 g. in 100 ml. of methanol, and further diluted if necessary) was determined at 215 mμ.[21] The percentage of conversion to pseudosapogenin was determined by dividing the specific absorption coefficient found experimentally by the corresponding value of the pure pseudosapogenin. In the case of the 188° pseudomerization of the 20-isosapogenins with omission of acetic anhydride, the product was, on completion of the heating period, dissolved directly in methanol and the ultraviolet absorption determined as above. The data for natural sapogenins are shown in Table I, for 20-iso and 20,22-isosapogenins in Table II. The results obtained by ultraviolet measurement were checked by paper chromatography. Whatman No. 4 paper, 12 by 18 inches, was impregnated with propylene glycol by dipping the sheets into a solution of 70% acetone–30% propylene glycol (v./v.). Approximately 200-microgram quantities of reaction products, pure pseudosapogenin or pure sapogenin were spotted on the paper. The mobile phase was a mixture of 25% benzene–75% cyclohexane (v./v.). Using a descending system with 12 × 18 inch circular tanks, the chromatograms were allowed to develop for 2 hours. The papers were dried and sprayed with 5% phosphomolybdic acid in ethanol.[22] The unreacted sapogenin moved much further than the pseudosapogenin so that mixtures were easily separated. The results qualitatively were in line with the data given in Tables I and II. In addition, there was no evidence to indicate that products other than pseudosapogenin or unreacted sapogenins were formed.

(b) Macro Procedure.—Sarsasapogenin acetate, 20.0 g., and 50.0 ml. of acetic anhydride containing a trace of glacial acetic acid were placed in a Pyrex, 250 ml. 24/40 ☿, round-bottom flask and the air flushed out with dry, oxygen-free nitrogen. The flask was then closed with a stopper previously ground with carborundum to make an air-tight seal. The stopper was held in place by a strong spring attached to a collar secured to the neck of the flask. The heating system consisted of a 2-liter resin flask containing 1 liter of a butyl Cellosolve fraction boiling at 170°. By means of a metal weight the reaction flask was completely immersed in the heating-bath. The butyl Cellosolve was then heated to boiling and the reaction flask heated 4 hr.[23] The reaction flask was then removed, cooled and the acetic anhydride distilled *in vacuo*. The residue was refluxed for one-half hour in methanol containing 5% potassium hydroxide. After addition of two volumes of water, the precipitated pseudosarsasapogenin was filtered, washed and dried to give 18.2 g., m.p. 140–170°. Crystallization from ethyl acetate gave 15.6 g., m.p. 168–171° (lit.[24] gives m.p. 168–171°) yield 85.5%. Papergram analysis of the mother liquors indicated that there was considerable pseudosarsasapogenin left in these residues. In a similar manner 20.0 g. of smilagenin acetate, heated 17 hr. at 170°, gave 16.0 g. of crystals from ethyl acetate, m.p. 153–161° (lit.[24] gives m.p. 153–161°) yield 88%. Tigogenin acetate, 17 hr. at 170° gave 16.6 g. of crystals from methanol, m.p. 170–180° (lit.[21] gives m.p. 179–189°), yield 91.5%; diosgenin acetate, 17 hr. at 180°, gave 11.1 g. of crystals from aqueous methanol, m.p. 160–170° (lit.[21] gives double m.p. 157–163°, 174–177°) yield 62%, and 3.6 g. of a less pure fraction, m.p. 150–165°, yield 20.0%; hecogenin acetate, 17 hr. at 180°, gave 16.0 g. of crystals from ether, m.p. 190–195° (lit.[21] gives m.p. 190–191°), yield 88.0%.

PHILADELPHIA 18, PENNA.

(18) D. H. W. Dickson *et al.*, *Chemistry & Industry*, 692 (1954).

(19) To obtain consistent results at 170° it was necessary to use reagent grade acetic anhydride, 454.5 g., to which was added 0.5 ml. of glacial acetic acid.

(20) This was both a safety precaution and a convenient means of removing the tube from the heating-bath by means of a length of copper wire attached to the spiral.

(21) A. F. B. Cameron, R. M. Evans, J. C. Hamlet, J. S. Hunt, P. G. Jones and A. G. Long, *J. Chem. Soc.*, 2807 (1955).

(22) D. Kritchevsky and M. R. Kirk, *Arch. Biochem. and Biophys.*, **35**, 346 (1952).

(23) The optimum heating period will vary depending on how much acetic acid is originally present in the acetic anhydride. In some cases we found 2 hr. to be sufficient.

(24) I. Scheer, R. B. Kostic and E. Mosettig, THIS JOURNAL, **77**, 645 (1955).

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Synthesis of Racemic, Optically Active and Radioactive α-Lipoic Acids

BY D. S. ACKER AND W. J. WAYNE

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A convenient and versatile route to α-lipoic acids using the reaction of 6,8-dichloroöctanoic acid or its esters with sodium disulfide is described. The synthesis of DL-α-lipoic acid, (+)-α-lipoic acid and DL-α-lipoic acid-S_2^{35} is reported. The preparation of (+)-α-lipoic acid by resolution of DL-α-lipoic acid is described.

α-Lipoic acid, 1,2-dithiolane-3-valeric acid, has been recognized as a new cofactor involved in the biochemical decarboxylation of α-keto acids and as a growth factor for a variety of microörganisms.