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[CONTRIBUTION FROM THE EASTERN REGIONAL RESEARCH LABORATORY AND THE DEPARTMENT OF CHEMISTRY,
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Steroidal Sapogenins. LXIII. Chiapagenin, a New Normal (25 L) Δ^5 -12 β -Hydroxysapogenin²

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A new normal Δ^5 -12 β -hydroxysapogenin, for which the name chiapagenin is proposed, has been isolated from the tubers of *Dioscorea chiapasensis* Matuda.⁴ Unequivocal proof of structure was obtained by catalytic hydrogenation of chiapagenin followed by acidic isomerization at C₂₅ to give the known 5 α ,12 β -hydroxysapogenin rockogenin. Reduction of the known normal Δ^5 -12-ketosapogenin correllogenin with lithium in liquid ammonia containing methyl alcohol gave chiapagenin. Therefore the latter must be 12 β -hydroxyyamogenin.

During the course of investigations of the plant kingdom for steroidal sapogenins, a new sapogenin was isolated from *Dioscorea chiapasensis* Matuda⁴ and identified. The two sapogenins present in this plant were separated by chromatography. The first product eluted was identified as the Δ^5 -normal (25 L) sapogenin, yamogenin, by direct

comparison with an authentic sample. Further elution gave a more polar, obviously polyhydroxy, sapogenin. The melting points of the latter compound and its acetate did not correspond to any previously known sapogenin. The structure of the new sapogenin, for which the name chiapagenin is proposed, was established in the following manner. Analysis of the new sapogenin and its acetate showed two hydroxyl groups present and the typical C₂₇ skeleton found in steroidal sapogenins. The infrared spectrum of chiapagenin diacetate showed this sapogenin to have the normal spiroketal side chain^{5,6} with bands characteristic of Δ^5 -unsaturation.^{7,8} This latter feature of the molecule

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(5) M. E. Wall, C. R. Eddy, M. L. McClennan, and M. E. Klumpp, *Anal. Chem.*, **24**, 1337 (1952).

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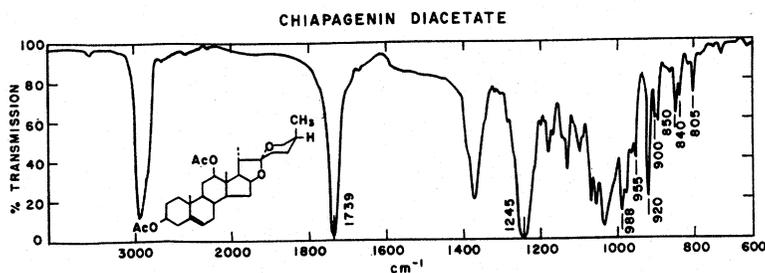


Figure 1

was confirmed by the high negative optical rotation.⁹

The entire structure of chiapagenin was established by two independent paths. Catalytic hydrogenation of chiapagenin gave a normal sapogenin

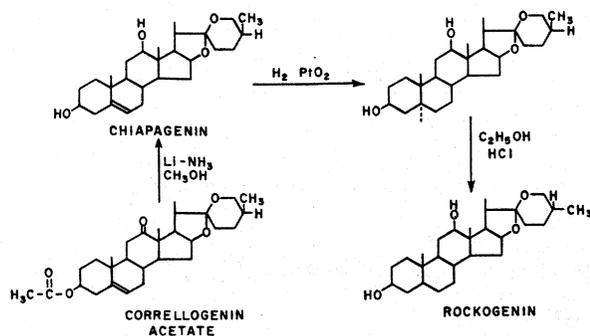


Figure 2

with the 5 α -ring A/B fusion. This compound was not further characterized but was subjected to prolonged heating at reflux temperature in ethanolic hydrochloric acid. This is the well known iso reaction which converts normal sapogenins with the axial methyl group to the corresponding isosapogenin (25 D) with the more stable equatorial methyl group.¹⁰ The crude reaction product was chromatographed and the major product, after acetylation, was shown to be rockogenin diacetate,^{11,12} identical with an authentic specimen. As the iso reaction inverts only the asymmetric center at C₂₅,¹⁰ and as under the experimental conditions used catalytic hydrogenation affects only the Δ^5 moiety,¹³ chiapagenin must be 12 β -hydroxyyamogenin.

The second independent proof of structure involved the normal Δ^5 -12-keto sapogenin correllogenin,¹⁴ which was reduced with lithium

metal in liquid ammonia in the presence of methanol.¹⁵ This reaction when applied to hindered ketones gives the most stable hydroxyl¹⁵ which, in the case of the 12-ketone, should be the equatorial 12 β -hydroxyl group. In accordance with this prediction, correllogenin acetate reduced in the above manner gave chiapagenin in excellent yield.

Steroidal sapogenins with a 12-hydroxyl group are of rare occurrence in nature. The only examples to date are rockogenin¹¹ and agavogenin,¹¹ which occur in a limited group of *Agave* species and are 5 α ,12 β -hydroxysapogenins with the iso side chain. The isolation from *D. chiapasensis* Matuda of chiapagenin represents the first case of a 12-hydroxy sapogenin to be found in this genus. Oxygenation at C₁₂ of any type is rare in the *Dioscorea*. In contrast to the *Agave* genus, where many species contain the 12-keto sapogenins hecogenin and/or manogenin,¹¹ the 12-ketones gentrogenin and correllogenin have to date been found only in *D. spiculiflora*.¹⁴

EXPERIMENTAL

Melting points were obtained with a Kofler¹⁶ micro melting-point apparatus. Optical rotations were determined in chloroform solution. Infrared spectra were obtained with a Perkin-Elmer Model 21 double beam spectrophotometer with sodium chloride prism and cells.

Isolation of chiapagenin. Tubers of *D. chiapasensis* Matuda were collected¹⁷ at the Finca Monte Bella of Sr. Cristobal above San Sebastian, Guatemala. The sample was received dry and was ground to give 5.2 kg. of dry material. This material was boiled, with stirring, for 1 hr. with two separate 5-gallon portions of 70% isopropyl alcohol, the extract being drawn off each time; 5 gallons of 90% isopropyl alcohol was used for the third extraction. These alcoholic extracts were combined and concentrated by distillation to 8.6 l. which was refluxed 4 hr. with 1.3 l. of concd. hydrochloric acid. The crude precipitate was filtered, washed with 10 l. of 5% sodium bicarbonate solution followed by 6 l. of water, and the solids dried in a forced-air oven. This material was extracted in a soxhlet extractor with 4 l. of heptane for 60 hr. The heptane solution of crude sapogenins was concentrated by distillation to 700 ml. and after standing at room tem-

(9) D. H. R. Barton and W. Klyne, *Chem. & Ind. (London)*, 755 (1948).

(10) For a comprehensive review of the iso reaction, cf. Fieser and Fieser, *Steroids*, Reinhold Pub. Corp., New York, 1959, pp. 818-22.

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(16) Specification of brand names of equipment and material used does not imply endorsement over similar commercial products.

(17) Tubers were collected by Dr. Ernest P. Imle, present address, Director of Research, American Cocoa Research Institute, 1741 K Street, N.W., Washington, D. C.

perature for several days 3.8 g. of crude crystalline material was deposited. This was chromatographed on a Florisil column using benzene as solvent. The benzene eluates gave 1.4 g. of a monohydroxy sapogenin. This material (0.2 g.) was refluxed for 30 min. in 5 ml. of acetic anhydride in pyridine (1:1). The solution was evaporated to dryness *in vacuo* and the residue recrystallized from methyl alcohol to give needles, m.p. 194–197°. These crystals were identified as yamogenin acetate by comparison of its infrared spectra with that of an authentic specimen. Elution with benzene-chloroform (1:1) and chloroform gave 1.8 g. of chiapagenin which, after recrystallization from methyl alcohol, melted at 249–251°, $[\alpha]_D^{25} -126^\circ$.

Anal. Calcd. for $C_{27}H_{42}O_4$: C, 75.31; H, 9.83. Found: C, 75.25; H, 10.06.

Acetylation of 0.2 g. of chiapagenin, as in the previously described manner, and recrystallization of the product from methyl alcohol yielded 0.14 g. rectangular needles, m.p. 191–193°, $[\alpha]_D^{25} -127^\circ$. The infrared spectrum shows strong bands at 1739 and 1245 cm^{-1} (acetate carbonyl and —C—O—C— stretching respectively) and bands at 988 and 920 (strong), 900 and 850 (weak) cm^{-1} , all four bands attributed to the normal spiroketal system. Bands at 840 and 805 cm^{-1} are due to Δ^5 unsaturation.

Anal. Calcd. for $C_{27}H_{40}O_6$: C, 72.34; H, 9.01. Found: C, 72.65; H, 9.22.

Conversion of chiapagenin to rockogenin. A 0.272-g. sample of chiapagenin was dissolved in a solution of 100 ml. of a 5% solution of glacial acetic acid in ethyl alcohol to which was then added 0.272 g. of platinum oxide. The mixture was hydrogenated at 3 atm. for 21 hr. at room temperature. The platinum was filtered and all the solvent removed *in vacuo*. Infrared analysis showed the double bond had been saturated (absence of 805 and 840 cm^{-1} bands). The white amorphous looking material which resulted was dissolved in 47 ml. of absolute ethyl alcohol. Concentrated hydrochloric acid (7 ml.) was then added and the resulting solution was refluxed 48 hr. Solvent was removed *in vacuo* and the residue chromatographed on a Florisil column using benzene as solvent. Elution with chloroform gave a glass

which on crystallization from acetone yielded 60 mg. of crystals. This crystalline fraction was acetylated in the usual manner and gave crystals from methyl alcohol, m.p. 200–203. The infrared spectrum was identical with that of authentic rockogenin diacetate.

Conversion of corrollogenin to chiapagenin. A 0.33-g. sample of corrollogenin acetate was dissolved in a mixture of 5 ml. of anhydrous ether and 5 ml. of anhydrous tetrahydrofuran in a 100 ml. two-necked round bottomed flask. The solution was added, with continued stirring, to a solution of liquid ammonia (25 ml.) containing 5 ml. of methyl alcohol. Lithium wire, 0.2 g., cut in small pieces, was added rapidly. After 5 min. 2.0 g. of ammonium chloride was added and the evaporation of ammonia was hastened by a warm water bath. Water (50 ml.) was then added and the aqueous slurry extracted twice with 20-ml. portions of methylene chloride. The solvents were evaporated and the residue acetylated, using the procedure previously described, to yield 0.21 g. of short rectangular needles from methyl alcohol, m.p. 191–193°, and an infrared spectrum identical with that of natural chiapagenin diacetate.

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