simplified variation of the procedure used by Wolfrom and Wood,[9] 5.5 g. of the recrystallized material was dissolved in 65 ml. of glacial acetic acid containing a trace each of copper powder and cupric acetate, as catalysts. As the solution was heated to just below its boiling point, a sudden and copious evolution of nitrogen occurred; after this had subsided, the solution was refluxed for 5 min., cooled, and concentrated to a sirup that contained, presumably, principally *keto*-D-*erythro*-L-*gluco*-nonulose heptaacetate. The sirupy product was cooled to 0° and deacetylated by adding a solution of 18.5 g. of barium hydroxide octahydrate in 110 ml. of water also at 0°, and stirring for 0.5 hr. to dissolve the sirup and then 3 hr. more at 0°. Barium ions were precipitated by the addition of oxalic acid, the barium oxalate was removed by filtration through Celite, and the filtrate was deionized completely by passing it through columns of Dowex 50 and Duolite A-4 ion-exchange resins. The eluate was concentrated to a sirup that was dissolved in 10 ml. of methanol and the nonulose precipitated by the addition of ethanol. After the precipitate had been freed from solvents *in vacuo* the nonulose remained as a dry, white, amorphous, hygroscopic powder weighing 2.17 g. and showing $[\alpha]^{20}D$ −47.2° in water (*c* 18); Wolfrom and Wood[9] reported $[\alpha]^{23}D$ also −47.2° in water (*c* 1.24). Paper chromatography carried out with solvents A, B, C, and D for periods of 4, 6, 5, and 1 days, respectively, failed to show any difference between the mobilities of the synthetic and the first avocado nonulose. The infrared spectra of the two nonuloses, as films from methanol on sodium chloride plates, had absorption bands at the same wave length and gave a further indication of the identity of the two sugars.

Preparation and Comparison of the D-*erythro*-L-*gluco*-Nonose 2,5-Dichlorophenylosazones from the First Avocado Nonulose and from the Synthetic Nonulose.—A 60-mg. sample of the avocado nonulose was refluxed with 250 mg. of 2,5-dichlorophenylhydrazine in 5 ml. of absolute ethanol containing 0.5 ml. of glacial acetic acid for 15 hr. on the steam bath. The osazone crystallized in clusters of fine, yellow needles from the boiling solution and, after being cooled, filtered, washed with ethanol and methanol, and dried, it weighed 58 mg.; m.p. 244–246° dec. The product appeared to be only sparingly soluble in the usual organic solvents and so was not recrystallized.

The 2,5-dichlorophenylosazone of the synthetic nonulose was obtained in the same manner; m.p. 248–250° dec. A mixture of the two osazones melted at 244–248° dec. The infrared spectra of the two osazones in Nujol mull also confirmed their identity.

Anal. Calcd. for $C_{21}H_{24}Cl_4N_4O_7$: C, 43.02; H, 4.13; Cl, 24.19; N, 9.56. Found (found avocado nonulose): C, 43.41; H, 4.40; Cl, 24.80; N, 9.50. Found (from synthetic nonulose): C, 43.29; H, 4.88; Cl, 23.57; N, 9.36.

Acknowledgment.—The authors wish to thank Mr. Harold G. McCann and his associates of the Analytical Services Unit of this laboratory for obtaining the infrared spectra and the elemental analyses.

Terpenoids. LIII.[1a] Demonstration of Ring Conformational Changes in Triterpenes of the β-Amyrin Class Isolated from *Stryphnodendron coriaceum*

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Acid hydrolysis of the saponins from *S. coriaceum* leads to a series of novel triterpenoid sapogenins, two of them—named *Stryphnodendron* sapogenins B and F—having been obtained in pure form. Mass spectrometry pointed towards an amyrin skeleton and chemical conversion demonstrated that B is the lactone of the cactus triterpene machaerinic acid, F being 2α-hydroxy-B. In contrast to all of the other known members of the α- or β-amyrin series, sapogenins B and F are readily hydrogenated, this unprecedented behavior being rationalized by the conformational alteration of rings D and E associated with lactone formation. In agreement with this conclusion is the observation that opening of the lactone ring restores the "nonreducibility" of the 12–13 double bond. Caution has to be exercised, therefore, in utilizing this criterion for excluding membership of an unknown triterpene in the α- or β-amyrin class.

Considerable mortality of cattle is registered during droughts in some parts of Brazil's Northeast, following ingestion of the bean pods of the plant *Stryphnodendron coriaceum* Benth. (family *Leguminosae Mimosacaea*). The hepatic and renal parenchyma of the animals are attacked and severe photosensitized lesions of the skin appear.[1b] These facts prompted chemical investigation of the plant at the Instituto de Quimica Agricola in Rio de Janeiro.[1b]

Extraction of the dried bean pods of *Stryphnodendron coriaceum* with ethanol afforded a considerable quantity of saponins. In this work no attempt was made towards their separation. The crude product was hydrolyzed and afforded a complex mixture of polyoxygenated triterpenic sapogenins. Separation of the individual sapogenins was effected by column chromatography and the compounds named A, B, C, etc.,—in the order of their elution.

Sapogenin B, m.p. 240–243°, $[\alpha]D$ −16°, had the empirical formula $C_{30}H_{46}O_3$, confirmed by mass spectrometry. The infrared spectrum showed a hydroxyl band at 3400 cm.$^{-1}$ and a band at 1776 cm.$^{-1}$, attributable to a γ-lactone. The nature of the three oxygen atoms was thus accounted for. The 60-Mc. n.m.r. spectrum of B showed signals corresponding to one vinyl proton at 5.55 δ (complex structure), one proton at 4.12 δ appearing as a neat doublet with J = 6 c.p.s. (attributable to the CH–O–CO lactonic proton), 9 protons at 1.02 δ (3 methyl groups), and 12 protons at 1.10, 0.90, 0.82 and 0.75 δ (4 methyl groups appearing as singlets). The mass spectrum, besides the molecular ion, showed prominent peaks at m/e 201 and 246.

The secondary nature of the hydroxyl group was readily confirmed by oxidation with Jones' reagent[2] to B ketone (VII), $C_{30}H_{44}O_3$, while the presence of the lactone was established by hydrolysis of B to B acid (III), $C_{30}H_{48}O_4$. The acid III was esterified with diazomethane, giving B methyl ester (IV), $C_{31}H_{50}O_4$, which in turn was diacetylated to diacetoxy B methyl ester (V), $C_{35}H_{54}O_6$; furthermore, IV also could be

(1) (a) Paper LII, L. Novotny, J. Jizba, V. Herout, F. Sorm, L. H. Zalkow, S. Hu, and C. Djerassi, *Tetrahedron*, **19**, 1101 (1963); (b) J. Döbereiner and C. F. Cesare Canella, *Bol. Soc. Bras. Med. Vet.*, **24**, 49 (1956).

(2) K. Bowden, I. M. Heilbron, E. R. H. Jones, and B. C. L. Weedon, *J. Chem. Soc.*, 39 (1946).

TABLE I

PRINCIPAL MASS SPECTRAL PEAKS OF *Stryphnodendron* SAPOGENINS B AND F AND THEIR DERIVATIVES

Substance	Type	R_1	R_2	R_3	R_4	R_5	*m/e* 201	*m/e* 246	*m/e* 260
B (I)	a′	OH			H	H	b″	b′	
B Ketone (VII)	a′	=O			H	H	b″	b′	
B Methyl ester (IV)	a	OH	OH	COOMe	H	H	b-(H_2O + R_3)	b′[a]	b-H_2O
Diacetoxy B methyl ester (V)	a	AcO	AcO	COOMe	H	H	b-(AcOH + R_3)		b-AcOH
F (X)	a′	OH			H	OH	b″	b′	
F Diacetate (XI)	a′	AcO			H	AcO	b″	b′	
F Diketone (XII)	a′	=O			H	=O	b″	b′	

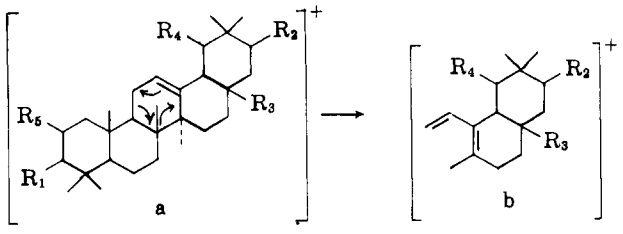
[a] Formation of this fragment is presumably due to relactonization upon heating (see ref. 6).

oxidized with Jones' reagent to "diketo B methyl ester" (VI), $C_{31}H_{46}O_4$. On dehydration with phosphorus oxychloride in pyridine, B afforded dehydro B (IX), $C_{30}H_{44}O_2$, m.p. 300–301°.

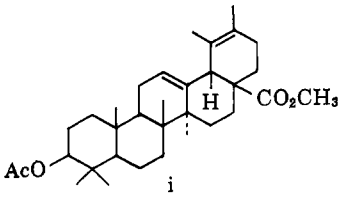
Sapogenin B was hydrogenated with ease at room temperature and atmospheric pressure with either platinum oxide or palladium on charcoal in acetic acid. The uptake of hydrogen corresponded to 1.03 moles and was confirmed by the mass spectrum of the resulting dihydro B ($C_{30}H_{48}O_3$). This compound showed no further sign of unsaturation (disappearance of the vinyl proton in the n.m.r. spectrum, lack of ultraviolet end absorption, negative test with tetranitromethane); sapogenin B must, therefore, be hexacyclic.

The ease of hydrogenation of B would seem to exclude a Δ^{12}-α- or β-amyrin skeleton, where the double bond is known to be highly unreactive. β-Amyrin, for instance, is not reduced at 280° and eighty atmospheres of hydrogen in acetic acid or ethyl acetate solution in the presence of platinum black or platinum oxide.[3] However, the fragmentation pattern appearing in the mass spectrum of B and its derivatives pointed strongly to such a Δ^{12}-amyrin skeleton (see Table I).

It has been established[4] recently that the molecular ion of compounds of the type a undergoes a reverse Diels Alder fragmentation to furnish a characteristic ion b, corresponding to rings D and E. The peak is generally followed by a second peak corresponding to b minus the C-17 substituent R_3.

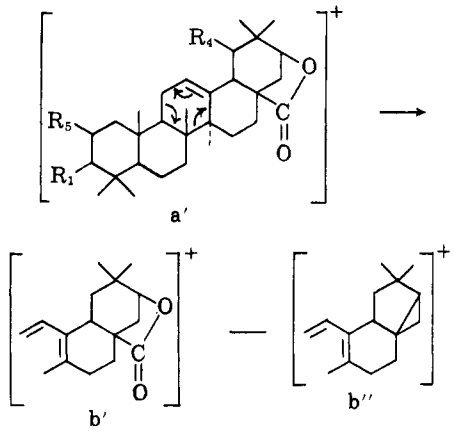


(3) L. Ruzicka, H. W. Huyser, M. Pfeiffer, and C. F. Seidel, *Ann.*, **471**, 21 (1929). However, during the preparation of this manuscript, there appeared an article by D. H. R. Barton, H. T. Cheung, P. J. L. Daniels, K. G. Lewis, and J. F. McGhie, *J. Chem. Soc.*, 5163 (1962), in which is reported the hydrogenation (platinum oxide in acetic acid, 20 hr.) of the novel triterpene methyl tomentosolate acetate (i) in 13% yield to the corresponding tetrahydro derivative.



(4) C. Djerassi, H. Budzikiewicz, and J. M. Wilson, *Tetrahedron Letters*, 263 (1962).

The mass spectrum of B methyl ester showed very strong peaks at *m/e* 260 and 201. This was highly reminiscent of the fragmentation pattern[4] of methyl siaresinolate 3-acetate (a, R_1 = AcO, R_3 = $COOCH_3$, R_4 = OH, R_2 = R_5 = H), which showed a strong *m/e* 278 peak (b ion), followed by peaks at *m/e* 260 (b-H_2O) and *m/e* 201 (b-H_2O-R_3). If we assume that B methyl ester has a β-amyrin skeleton with R_3 = $COOCH_3$ and R_1 = R_2 = OH, we do not find the expected b ion at *m/e* 278 but the prominent peaks at *m/e* 260 and 201 can be interpreted as (b-H_2O) and (b-H_2O-R_3), respectively, in the same manner as for methyl siaresinolate acetate.



If one formulates sapogenin B as a′ (R_1 = OH, R_4 = R_5 = H), then the predictable b′ and b″ fragments are found where expected, as can be seen in Table I. Species b″ is formally drawn as a cyclopropane, but it may well exist instead as the rearranged olefin.

All these indications give strong support for an amyrin type skeleton, despite the unprecedented hydrogenation behavior of B. Indeed, chemical proof of the structure of B showed it to belong to the β-amyrin group. It is logical to suppose that the hydroxyl group is of the 3β-type as in most triterpenes, an assumption supported by the n.m.r. signal (195 c.p.s.) of the 3α-axial proton. Mass spectrometry favored the location of the lactone bridge in rings D and E, for instance between carbons 17 and 21. Direct comparison of various derivatives showed that sapogenin B was in fact the lactone of the already reported[5] machaerinic acid (III), as can be seen in Table II.

The lactone of machaerinic acid until now had not been isolated from a natural source. However, it had been shown[6] that methyl machaerinate on heating generated a lactone which was not isolated. This observa-

(5) C. Djerassi and A. E. Lippman, *J. Am. Chem. Soc.*, **77**, 1825 (1955).
(6) C. Djerassi and J. S. Mills, *ibid.*, **80**, 1236 (9158).

TABLE II

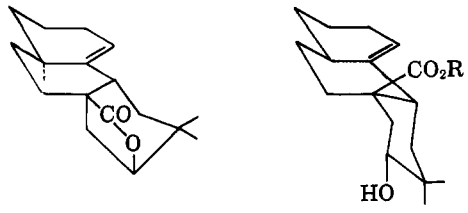
COMPARISON OF SAPOGENIN B AND MACHAERINIC ACID DERIVATIVES

	M.p.,[a] °C	$[\alpha]_D$
B Methyl ester	230–233	−79°
Methyl machaerinate (IV)[b]	232–234	+76°
Diacetoxy B methyl ester	268–277	+81°
Diacetoxy methyl machaerinate (V)[b]	278–280	+86°
Diketo B methyl ester	180–183	+41°
Diketo methyl machaerinate (VI)[b]	188–191	+43°

[a] The three pairs of compounds showed no depression of the melting poin on admixture. [b] See ref. 5.

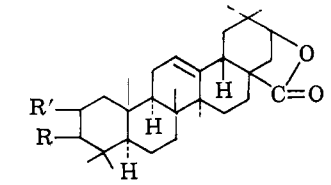
tion offered a plausible explanation for the *m/e* 246 fragment in the mass spectrum of B methyl ester (methyl machaerinate) (see Table I).

From a model it can be seen that the formation of the lactone bridge requires severe modifications of the conformations of both rings D and E, a conclusion which had been reached earlier[6] on the basis of saponification

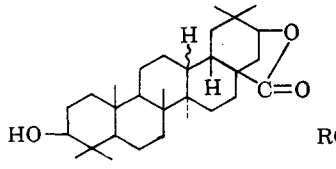


rate studies in the treleasegenic acid series. That this deformation, with consequent reduced steric hindrance for catalyst adsorption, is the responsible factor for the ease of hydrogenation of B was demonstrated by the fact that methyl machaerinate (IV) was recovered unchanged under hydrogenation conditions where B (I) was reduced rapidly. A boat conformation of ring E was also indicated by the fact that the lactonic proton at C-21 appeared as a sharp doublet in the n.m.r. spectrum of B being thus coupled with only one of the two neighboring protons at C-22. In the boat form, the angle between the α C-21 proton and the α C-22 proton is very nearly 90° and their coupling constant must be equal to zero or at least very small.[7]

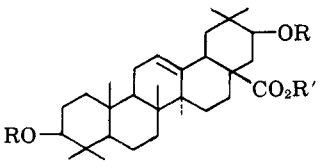
Another manifestation of this conformational change is the observation that dihydro-B acid relactonizes immediately under conditions where B acid [= machaerinic acid (III)] is perfectly stable, thus suggesting the 13α-stereochemistry in the dihydrolactone II.



I, R=OH; R'=H
X, R=R'=OH
XI, R=R'=OAc



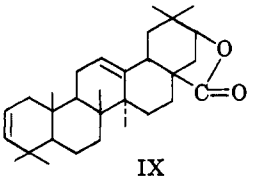
II



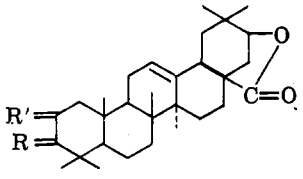
III, R=R'=H
IV, R=H; R'=CH₃
V, R=Ac; R'=CH₃

Sapogenin F, m.p. 265–267° $[\alpha]_D$ +5°, had the empirical formula $C_{30}H_{46}O_4$. Its infrared spectrum showed a strong band at 3420 cm.$^{-1}$ indicating the presence of one or more hydrogen-bonded hydroxyl groups and a band at 1770 cm.$^{-1}$ attributable to a γ-lactone. The n.m.r. spectrum contained signals for one vinyl proton at 5.58 δ, one proton at 4.13 δ (sharp doublet, J = 6), 9 protons at 1.05 δ (3 methyl groups), and 12 protons at 1.13, 1.00, 0.87 and 0.78 δ (4 methyl groups). All these signals appeared as singlets. The mass spectrum exhibited strong peaks at *m/e* 201 and 246 (see Table I). Sapogenin F gave a diacetate $C_{34}H_{50}O_6$, showing that two of the oxygens are present as hydroxyl groups, whereas the other two are part of a γ-lactone. Compound F was hydrogenated under the same conditions as B, absorbing 0.96 mole of hydrogen. All these features suggested a close connection between B and F and led to the obvious working hypothesis that F was x-hydroxy B.

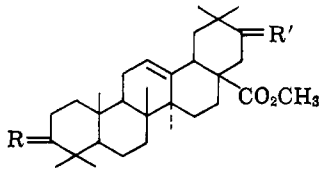
Oxidation with Jones' reagent[2] gave a slightly yellowish diketone (XII) which exhibited ultraviolet absorption at 270 mμ in neutral solution and at 240 as well as 315 mμ in alkaline solution, characteristic of an α-diketone. The presence of an α-glycol system in F was corroborated by the fact that the substance consumed 1.08 moles of periodate and also formed easily an acetonide.



IX



VII, R=O; R'=H₂
XII, R=R'=O



VI, R=R'=O
VIII, R=H₂; R'= ···H (OH)

Diketo F (XII) was reduced by the Wolff–Kishner reaction to an acid that on immediate methylation with diazomethane afforded bisdeoxy F methyl ester. By similar treatment, keto B (VII) afforded deoxy B methyl ester (VIII), and direct comparison showed the identity of the two specimens, thus demonstrating that F had the same carbon skeleton as B, with the γ-lactone ring in the same position.

Conversion of F (X) to the ditosylate and treatment with sodium iodide in acetone at 100° gave in good yield dehydro B (IX), thus proving that the two hydroxyl groups in F were attached to C-2 and C-3. Of the four stereochemical possibilities, the diaxial 2β,3α-orientation could be discarded because acetonide formation would be impossible in this case. Dehydro B (IX), when treated on a microscale with osmium tetroxide, afforded a diol, which was clearly different from F (X) as determined by thinlayer chromatography. Since the osmylation product must possess

(7) For leading references to the Karplus equation see H. Conroy, "Advances in Organic Chemistry," Vol. II, R. A. Raphael, E. C. Taylor, and H. Wynberg, Ed., Interscience Publishers, Inc., New York, N. Y., 1960, p. 310, as well as R. J. Abraham and J. S. E. Holker, *J. Chem. Soc.*, 806 (1963).

the 2α,3α-orientation, this system also is excluded. The formation of the Δ²-compound from the disulfonyl ester has been reported[8] in the steroid series to work only for the 2α,3α- and 2α,3β-isomer. It has been suggested that the failure of the 2β,3β- and 2β,3α-steroid esters to react is associated with steric hindrance of the axial 2β ester with the angular methyl group.[9] If this is correct, the steric hindrance of a 2β-substituent in an amyrin type triterpene must be even greater, because of the presence of the additional axial methyl group at C-4. The 2β,3α-system being discarded, this leads to the assignment of a 2α,3β-stereochemistry to the glycol system of F, *provided ring A exists largely in the chair conformation.* A 2β,3β-isomer would not be excluded if ring A assumes a boat-like form.

At first sight, it seems somewhat contradictory that the *trans*-2α,3β-glycol system of F affords an acetonide, since only steroidal *cis*-glycols have been reported[8,10] to form such derivatives. In fact, there is no *a priori* reason why acetonide production from a *trans* diequatorial α-glycol system should be rejected, since the distance between two *trans* diequatorial hydroxyl groups is the same as the distance between two *cis* axial–equatorial ones.[11]

That sapogenin F has in fact the 2α,3β-stereochemistry is confirmed by the n.m.r. spectrum of its diacetate XI, where the protons at C-2 and C-3 give rise to a doublet (J = 11 c.p.s.) centered at 4.70 δ. The value of the coupling constant shows[7] that the two pertinent protons are at an angle close to 180° (*trans* diaxial system) in agreement with the suggested 2α,3β-configuration.

Experimental[12]

Extraction.—The dried bean pods of *Stryphnodendron coriaceum* (60 kg.) were ground to a powder and continuously extracted with 95% alcohol for 4 days. Concentration of the extract under reduced pressure at 50° gave 12 l. of a brown sirup. By hydrolysis of the 12 l. of extract in 24 l. of alcohol and 3.5 l. of concentrated hydrochloric acid for 2 hr. at 80°, followed by dilution with water and filtration, there was obtained 2.5 kg. of brown powder. This was suspended in boiling benzene and filtered, giving 700 g. of benzene-soluble sapogenins. Examination of this mixture on silica gel chromatoplates showed the presence of six major coconstituents and smaller amounts of many more.

Isolation of Sapogenins B and F.—A portion (48 g.) of the sapogenin mixture was chromatographed on a 350-g. column of alumina, using as eluent a gradient of solvents, from petroleum ether to ether. All fractions were examined on chromatoplates, checking them against the original mixture. The fractions eluted with 40 to 50% ether in petroleum ether, containing most of B, were combined and rechromatographed using the same procedure, affording practically pure B (I). Several recrystallizations from methanol–chloroform afforded 6 g. (0.14% yield based on dried bean pods) of pure B, m.p. 240–243°, $[\alpha]_D$ −16°.

Anal. Calcd. for $C_{30}H_{46}O_3$: C, 79.24; H, 10.20; mol. wt., 454.7. Found: C, 79.40; H, 10.31; mol. wt., 454 (mass spec.).

The first fractions eluted with pure ether contained most of F, and were combined and re-chromatographed in the same manner. Several recrystallizations from ethanol–petroleum ether afforded 2.5 g. (0.06% yield) of pure F (X), m.p. 265–267°, $[\alpha]_D$ +5°.

Anal. Calcd. for $C_{30}H_{46}O_4$: C, 76.55; H, 9.87. Found: C, 76.14; H, 9.82.

Catalytic Hydrogenation of Sapogenin B.—Sapogenin B (I) was easily hydrogenated in acetic acid solution with platinum oxide or 10% palladium on charcoal at room temperature and atmospheric pressure. Hydrogen uptake corresponding to 1.03 moles was complete within 2 hr. and the resulting dihydro B (II), obtained in nearly quantitative yield, after crystallization from ethyl acetate had m.p. 325–326°.

Anal. Calcd. for $C_{30}H_{48}O_3$: C, 78.89; H, 10.59; O, 10.51; mol. wt., 456.7. Found: C, 78.74; H, 10.61; O, 10.69; mol. wt., 456 (mass spec.).

Hydrolysis of Sapogenin B.—Sapogenin B (I) (400 mg.) was hydrolyzed by heating under reflux with 30 ml. of 5% ethanolic sodium hydroxide solution for 1.5 hr. On dilution with water, the sodium salt crystallized as long needles which were filtered. The acid was regenerated by treatment with dilute hydrochloric acid and recrystallized from ethanol–chloroform to provide in 93% yield **machaerinic acid (III)** with m.p. 295–305°.

Anal. Calcd. for $C_{30}H_{48}O_4$: C, 76.22; H, 10.24; O, 13.54. Found: C, 75.88; H, 10.21; O, 13.61.

A sample (100 mg.) of the acid (III) was suspended in 8 ml. of ether and an ethereal solution of approximately 50 mg. of diazomethane was added. The mixture was left overnight, then evaporated. Recrystallization of the residue from chloroform–methanol afforded in nearly quantitative yield **methyl machaerinate (IV)**, m.p. 230–233°, $[\alpha]_D$ +79°.

Anal. Calcd. for $C_{31}H_{50}O_4$: C, 76.50; H, 10.36; O, 13.15. Found: C, 76.76; H, 9.92; O, 13.06.

Acetylation of 50 mg. of B methyl ester (IV) was effected overnight with acetic anhydride–pyridine at room temperature. The mixture was poured on ice and the precipitate collected. Recrystallization from methanol–chloroform afforded in 88% yield **methyl machaerinate diacetate (V)**, m.p. 268–277°, $[\alpha]_D$ +81°.

Anal. Calcd. for $C_{35}H_{54}O_6$: C, 73.64; H, 9.54. Found: C, 73.96; H, 9.65.

The pure B methyl ester (IV) (82 mg.) was dissolved in 3 ml. of acetone and stirred at room temperature. Jones' reagent[2] was slowly added until the brown coloration persisted, then the reaction mixture was stirred for 1.5 min. Water was added, the precipitate extracted with chloroform, dried with magnesium sulfate, filtered, and evaporated. Recrystallization from chloroform–methanol afforded 53 mg. of **diketo methyl machaerinate (VI)**, m.p. 180–183°, $[\alpha]_D$ +41°.

Anal. Calcd. for $C_{31}H_{46}O_4$: C, 77.13; H, 9.61. Found: C, 76.90; H, 9.53.

Oxidation of Sapogenin B.—Treatment of 120 mg. of pure B (I) as described earlier and two recrystallizations from chloroform–methanol afforded keto B (VII), m.p. 294–294.5° (92 mg.).

Anal. Calcd. for $C_{30}H_{44}O_3$: C, 79.60; H, 9.80; O, 10.60. Found: C, 79.71; H, 9.68; O, 10.83.

Methyl 3-Deoxymachaerinate (VIII) from Sapogenin B.—The ketone VII (92 mg.) was dissolved in the minimum amount of ethanol; 5 ml. of diethyleneglycol was added and the alcohol distilled out of the solution, followed by the addition of 0.9 ml. of anhydrous hydrazine and gentle refluxing for 1 hr. The excess hydrazine was then distilled, the flask cooled, and 0.45 g. of powdered potassium hydroxide added. The reaction mixture was heated at 200° for 2 hr., then cooled. Water was added and the precipitate filtered and repeatedly washed with distilled water. Purification of the acid obtained proved difficult and the crude compound, dissolved in ether, was esterified with diazomethane. Evaporation left a yellow resin which was chromatographed on a short column of alumina, using a gradient of solvents from benzene to ether. The product (19 mg.) was eluted with about 40% of ether. Three recrystallizations from methanol–chloroform afforded pure deoxy B methyl ester (VIII), m.p. 183–189°, $[\alpha]_D$ +81°.

Anal. Calcd. for $C_{31}H_{50}O_3$: C, 79.10; H, 10.71. Found: C, 79.42; H, 10.96.

Dehydration of Sapogenin B.—A solution of 100 mg. of B (I) in 10 ml. of anhydrous pyridine and 0.8 ml. of phosphorus oxychloride was heated under reflux for 1 hr. After cooling, water was added and the precipitate extracted with chloroform, dried with magnesium sulfate, filtered, and evaporated. The resulting slightly yellow resin was chromatographed on a short column of alumina, using a gradient of solvents from benzene to ether.

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(10) J. Herran, G. Rosenkranz, and F. Sondheimer, *J. Am. Chem. Soc.*, **76**, 5531 (1954).

(11) For acetonide, formation of nonsteroidal *trans*-glycols, see J. Boeseken and H. G. Derx, *Rec. trav. chim.*, **40**, 529 (1921), and S. J. Angyal and C. G. MacDonald, *J. Chem. Soc.*, 686 (1952).

(12) All melting points are uncorrected (recorded on a hot-stage microscope), while all rotations refer to 1.0% solutions in chloroform. All alumina used in this work is Alcoa F-20.

The first fractions afforded white crystals, which were recrystallized twice from methanol–chloroform giving 67 mg. of pure dehydro B (IX), m.p. 300.5–301°.

Anal. Calcd. for $C_{30}H_{44}O_2$: C, 82.51; H, 10.16. Found: C, 82.55; H, 10.14.

Alternatively, 105 mg. of F (X) was dissolved in 5 ml. of anhydrous pyridine and 0.3 g. of *p*-toluenesulfonylchloride was added. The reaction was left overnight at room temperature and water was added to the dark solution. The resulting precipitate was filtered, washed repeatedly with 20% sodium carbonate solution followed by water. The ditosylate was quite insoluble in most solvents and was not recrystallized, but appeared homogeneous on thin layer chromatography.

The crude tosylate was heated in a sealed tube at 100° for 1 hr. with 10 ml. of acetone and 250 mg. of sodium iodide. The tube was then cooled and the contents poured into water. The resulting precipitate was filtered and chromatographed on a short column of alumina using a gradient of solvents from benzene to ether. The first fractions afforded 19 mg. of a compound melting at 300–303° after two recrystallizations from methanol. This substance proved to be identical with dehydro B (IX) by mixture melting point determination and infrared comparison.

Acetylation of Sapogenin F.—A sample (100 mg.) of pure F (X) was acetylated with acetic anhydride–pyridine at room temperature. After 12 hr., water was added, the resulting precipitate filtered and repeatedly washed with water. Two recrystallizations from methanol–chloroform afforded in nearly quantitative yield F diacetate (XI), m.p. 222–226°, $[\alpha]_D$ −48°.

Anal. Calcd. for $C_{34}H_{50}O_6$: C, 76.61; H, 9.09. Found: C, 73.48; H, 9.23.

Oxidation of Sapogenin F.—The oxidation of 150 mg. of F (X) with Jones[2] reagent was performed as described before and the resulting compound (120 mg.) was repeatedly crystallized from various solvent mixtures but had a wide melting range above 150°. Diketo F (XII) had $[\alpha]_D$ +49° and showed only one large spot on chromatoplates in several solvents.

Anal. Calcd. for $C_{30}H_{42}O_4$: C, 77.21; H, 9.07. Found: C, 77.19; H, 9.13.

A portion (100 mg.) of diketo F (XII) was reduced and methylated as described before for the ketone VII. This procedure afforded bisdeoxy F methyl ester, m.p. 182–187°, $[\alpha]_D$ +81°, which was shown to be identical with deoxy B methyl ester (VIII) (methyl 3-deoxymachaerinate), m.p. 183–189°, $[\alpha]_D$ +81°, by mixture melting point determination and thin layer chromatography.

Sapogenin F Acetonide.—To a solution of 50 mg. of F (X) in 5 ml. of anhydrous acetone was added one drop of concentrated sulfuric acid and the reaction left overnight at room temperature. The course of the reaction can be followed conveniently by thin layer chromatography on silica gel using 3% ethyl acetate in benzene. Under these conditions, the unchanged sapogenin does not migrate, while the acetonide moves near the solvent front. The reaction mixture was concentrated and the acetonide precipitated by the addition of pentane. Attempted recrystallization normally resulted in reformation of the parent diol.

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Anthocyanins and Related Compounds. II. Structural Transformations of Some Anhydro Bases

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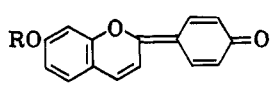
The spectra of anhydro bases derived from 4′,7-dihydroxyflavylium salts confirm a 7-keto structure for these compounds. At pH < 7 anhydro bases unsubstituted in the 3-positions rapidly hydrolyze to *cis*-2-hydroxychalcones. At about pH 9 and pH 12 spectral evidence suggests that anhydro bases derived from monohydroxyflavylium salts are hydrolyzed to yield *trans* and *cis* forms of ionized chalcones, respectively.

In aqueous solutions at pH > 4, anthocyanins and flavylium salts with at least one free hydroxyl in the 5-, 7-, 2′- or 4′- position lose a proton to form highly colored, labile anhydro bases. Since the pH of the cell sap of most plants[1] is in the range 4–6 it would seem probable that anhydro bases play a predominant role as reactive species in irreversible anthocyanin degradations in plant extracts. It is somewhat surprising, therefore, that the structures and transformation products of anhydro bases have not been more extensively investigated. Thus, although a 4′-keto structure of type I has been assigned arbitrarily to the anhydro bases derived from natural anthocyanins and other 4′,7-dihydroxyflavylium compounds,[2] experimental evidence in support of this structure has not been reported. Furthermore, while it is well established that in strongly alkaline (*e.g.*, sodium hydroxide) solutions anhydro bases are rapidly hydrolyzed to ionized chalcones,[3] the products formed at intermediate pH's are not, in many cases, known with certainty.

The anhydro base derived from a 4′,7-dihydroxyflavylium salt could conceivably arise by loss of a proton from either the 4′- or the 7-hydroxyl, resulting in structure I or II (R = H). Comparison of this



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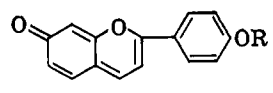

II

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