Anal. Calcd. for $C_8H_{15}N$: C, 76.74; H, 12.08; N, 11.19. Found: C, 76.26; H, 12.22; N, 11.00.

1,4,4-Trimethyl-Δ^1-tetrahydropyridinium perchlorate formed from the base above in ethanol–ether was recrystallized from acetone–ether, colorless prisms, m.p. 88–89°.

Anal. Calcd. for $C_8H_{16}ClNO_4$: C, 42.57; H, 7.15; N, 6.21. Found: C, 42.70; H, 7.37; N, 5.99.

1,4,4-Trimethyl-Δ^1-tetrahydropyridinium picrate crystallized from aqueous ethanol as yellow prisms, m.p. 180° with decomposition (unsharp), infrared maximum (mull) at 1700 cm.$^{-1}$.

Anal. Calcd. for $C_{14}H_{18}N_4O_7$: C, 47.45; H, 5.12; N, 15.81. Found: C, 47.35; H, 5.25; N, 15.82.

2-Cyano-1,4,4-trimethylpiperidine.—The crude perchlorate salt from 4.5 g. (0.036 mole) of free base in water was treated with excess potassium cyanide following the usual procedure to give 4.6 g. (85%) of 2-cyano-1,4,4-trimethylpiperidine, b.p. 103° (18 mm.), $n^{25}D$ 1.4557, infrared maxima at 2220 and 2240 cm.$^{-1}$ (mull).

Anal. Calcd. for $C_9H_{16}N_2$: C, 71.01; H, 10.60; N, 18.40. Found: C, 70.84; H, 10.89; N, 18.32.

Reduction of this compound with excess lithium aluminum hydride yielded 87% of **2-aminomethyl-1,4,4-trimethylpiperidine**, b.p. 99° (18 mm.), $n^{24}D$ 1.4693.

Anal. Calcd. for $C_9H_{20}N_2$: C, 69.17; H, 12.90; N, 17.93. Found: C, 68.84; H, 12.94; N, 17.42.

The dipicrate crystallized from aqueous ethanol as yellow prisms, m.p. 196–197° dec.

Anal. Calcd. for $C_{21}H_{26}N_8O_{14}$: C, 41.04; H, 4.26; N, 18.24. Found: C, 41.26; H, 4.37; N, 18.29.

1-Ethyl-4-methylpiperidine.—Hydrogenation of 75 g. (0.81 mole) of pure γ-picoline in 200 ml. of ethanol at 200° and 150 atmospheres using Raney nickel W-2 catalyst yielded 78 g. (76%) of 1-ethyl-4-methylpiperidine, b.p. 147°, $n^{24}D$ 1.4373.

Anal. Calcd. for $C_8H_{17}N$: C, 75.52; H, 13.47; N, 11.01. Found: C, 75.14; H, 13.50; N, 10.87.

The picrate crystallized from dilute ethanol as yellow needles, m.p. 155–156°.

Anal. Calcd. for $C_{14}H_{20}N_4O_7$: C, 47.19; H, 5.66; N, 15.73. Found: C, 47.32; H, 5.69; N, 15.55.

Mercuric Acetate Oxidation of 1-Ethyl-4-methylpiperidine.—The usual oxidation procedure yielded about 80% of a mixture of monomeric enamine and "dimeric" product. Redistilled **1-ethyl-4-methyl-Δ^2-tetrahydropyridine** boiled at 54–55° (18 mm.), $n^{24}D$ 1.4604, infrared maxima at 3020 and 1640 cm.$^{-1}$. The perchlorate salt was an oil.

Anal. Calcd. for $C_8H_{15}N$: C, 76.74; H, 12.08; N, 11.19. Found: C, 76.06; H, 12.58; N, 11.11.

Redistilled **1,1'-diethyl-4,4'-dimethyl-Δ^2-tetrahydroanabasine** boiled at 162° (18 mm.), $n^{25}D$ 1.4985, infrared maximum at 1650 cm.$^{-1}$, oily perchlorate.

Anal. Calcd. for $C_{16}H_{30}N_2$: C, 76.74; H, 12.08; N, 11.19. Found: C, 76.93; H, 12.31; N, 11.21.

URBANA, ILLINOIS

[JOINT CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF WAYNE STATE UNIVERSITY AND THE WESTERN UTILIZATION RESEARCH AND DEVELOPMENT DIVISION, AGRICULTURAL RESEARCH SERVICE, U. S. DEPARTMENT OF AGRICULTURE]

Terpenoids. XXXI.[1] The Structure and Stereochemistry of Medicagenic Acid[2]

BY CARL DJERASSI,[3] D. B. THOMAS,[3] A. L. LIVINGSTON[4] AND C. RAY THOMPSON[4]

RECEIVED JUNE 6, 1957

Medicagenic acid, a triterpenoid dihydroxy dicarboxylic acid from alfalfa, has been shown to be 2β,3β-dihydroxy-Δ^{12}-oleanene-23,28-dioic acid (Ia) by various degradations and by a direct correlation with arjunolic acid (VI). Quantitative lead tetraacetate oxidations have demonstrated that the glycol grouping in the related triterpenes asiatic acid, arjunolic acid, terminolic acid, barringtogenic acid and barringtogenol has the 2α,3β-orientation.

An examination[5] of the saponins of alfalfa (*Medicago sativa*) has led to the isolation of a new sapogenin, $C_{30}H_{46}O_6$, which was characterized as a dihydroxy dicarboxylic acid. The structure elucidation of this sapogenin—now named medicagenic acid—seemed particularly pertinent since the mixture of saponins which occurs in alfalfa is known to produce deleterious effects in chicks and ruminants[6] and since dicarboxylic acids of the pentacyclic triterpene series are very rare.[7] The present paper describes the establishment of the structure and stereochemistry of medicagenic acid.

Initial purification was accomplished *via* the diacetate Ib which was then methylated with diazomethane and chromatographed, since diacetoxy dimethyl medicagenate (Ic) lent itself particularly well to such purification and also crystallized readily.[8] Saponification of the diacetoxy dimethyl ester Ic with 5% potassium hydroxide or even with potassium carbonate solution led to the crystalline dimethyl medicagenate (Id).[9] Since the latter conditions are usually insufficient[10] to saponify the conventional 3β-acetoxy group of pentacyclic triterpenes, assumed to exist also in medicagenic acid, this indicated the presence of additional, activating substituents in ring A. In fact, treatment of dimethyl medicagenate (Id) with acetone in the presence of sulfuric acid[11] afforded an acetonide (II) which implied that the second hydroxyl group of the sapogenin had to be located at positions

(1) Paper XXX, A. Sandoval, A. Manjarrez, P. R. Leeming, G. H. Thomas and C. Djerassi, THIS JOURNAL, **79**, 4468 (1957).

(2) The work at Wayne State University was supported by a research grant (No. RG-3863) from the National Institutes of Health, U. S. Public Health Service.

(3) Wayne State University, Detroit, Michigan.

(4) Western Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture, Albany 10, Calif.

(5) E. D. Walter, G. R. Van Atta, C. R. Thompson and W. D. Maclay, THIS JOURNAL, **76**, 2271 (1954).

(6) For pertinent literature see ref. 5.

(7) At the time that this work was undertaken, the structure of only one such dicarboxylic acid—quinovic acid (H. Wieland and M. Erlenbach, *Ann.*, **453**, 83 (1927))—was known. Since that time, the structures of two additional triterpene dicarboxylic acids have been announced, namely, melaleucic acid (H. R. Arthur, A. R. H. Cole, K. J. L. Thieberg and D. E. White, *Chemistry & Industry*, 926 (1956)) and barringtogenic acid (R. Anantaraman and K. S. Madhavan Pillai, *J. Chem. Soc.*, 4369 (1956)).

(8) Several transformation products of medicagenic acid either did not crystallize or did so only with difficulty.

(9) The infrared spectrum of the dimethyl ester Id shows remarkable separation of the ester bands in Nujol mull (see Experimental) which seems to be due to hydrogen bonding, since this is not observed in the corresponding diacetate Ic.

(10) C. Djerassi, E. Farkas, A. J. Lemin, J. C. Collins and F. Walls, THIS JOURNAL, **76**, 2969 (1954).

(11) *Cf.* J. L. Beton, T. G. Halsall and E. R. H. Jones, *J. Chem. Soc.*, 2904 (1956).

2, 23 or 24, provided the other one was indeed present at C-3. A decision between these possibilities could be accomplished readily by lead tetraacetate oxidation of dimethyl medicagenate (Id), which resulted in the consumption of one equivalent of reagent, thus demonstrating the existence of a 1,2-glycol moiety in medicagenic acid.

In the stereochemically related steroidal sapogenin series, all four possible 2,3-dihydroxy isomers are known[12] and only the two *cis* glycols undergo acetonide formation. Furthermore, quantitative lead tetraacetate oxidation studies[13] have demonstrated that the rate of glycol cleavage differs to such an extent that it can be used readily for stereochemical assignment purposes. The value $k = 31 \times 10^{-3}$ l.-mole^{-1} sec. $^{-1}$ observed for dimethyl medicagenate (Id) is in excellent agreement with that reported[13] (31.9×10^{-3}) for the steriodal 2β,3β-glycol and significantly different from that (13.2×10^{-3}) recorded[13] for the 2α,3α-isomer.[14] It follows, therefore, that the two hydroxyl groups of medicagenic acid are located at positions 2β and 3β.

Attention was next turned toward locating the two carboxyl groups of medicagenic acid. The presence of one carboxylic function at C-17 was indicated by formation of an amorphous bromo lactone[5] (subsequently shown to be III) which showed infrared bands characteristic of a γ-lactone as well as of a carboxyl group. The production of a bromo lactone also required the presence of a 12-13 double bond, and this was confirmed by oxidation of diacetoxy dimethyl medicagenate (Ic) with selenium dioxide in glacial acetic acid leading to an amorphous diene. The ultraviolet absorption spectrum of the product was typical of $\Delta^{11,13(18)}$-dienes IV[15] thus establishing membership of medicagenic acid in the β-amyrin series since α-amyrins are essentially unreactive under those conditions.

A difference in the reactivity of the two carboxyl groups was noted when dimethyl medicagenate was boiled with 10% methanolic potassium hydroxide under conditions where methyl esters of C-17 carboxylic acids lacking activating groups[16] are not affected. The resulting product proved to be a monomethyl ester (subsequently shown to be Ie) which was best characterized as the diacetoxy monomethyl ester If. That the C-17 carboxylic acid methyl ester was indeed not affected by such treatment was demonstrated by the fact that the monomethyl ester Ie did not form a bromo lactone under the conditions employed successfully[5] with medicagenic acid (Ia) itself. The relative ease of saponification is best rationalized with positions 23,[17] 29 or 30, of which only the first alternative would yield a β-keto acid upon oxidation. Direct chromium trioxide oxidation of medicagenic acid (Ia) in acetone solution[18] produced a gum, but when this was warmed with alkali and then acidified, there was obtained a crystalline nor-acid Va with the typical ultraviolet absorption of the monoenol of an α-diketone (λ_{max}^{EtOH} 281 mμ, λ_{max}^{KOH} 329 mμ) and further characterized as the enol acetate Vb(λ_{max}^{EtOH} 247 mμ). Therefore the degradative evidence, summarized above, is only compatible with the expression Ia (2β,3β-dihydroxy-Δ^{12}-oleanene-23,28-dioic acid) for medicagenic acid, which also explains why no γ-lactone (23→ 2) is produced during the acid hydrolysis of the saponin since the two relevant substituents (2 and 23) are *trans* to each other.

In order to confirm the structural assignment, a direct correlation had to be accomplished with a triterpene of known constitution. For this purpose, arjunolic acid (VI)[19] was selected since its structure had been established by relating it to a degradation product of hederagenin. Oxidation of arjunolic acid (VI) in the manner described above for medicagenic acid (Ia) yielded the same diosphenol (Va) and derived enol acetate Vb as demonstrated by mixture melting point determination, infrared analysis and comparison of the ultraviolet absorption maxima and specific rotations.

The earlier reported structure proof[19] of arjunolic acid (VI) involved destruction of the asymmetric centers at C-2, C-3 and C-4 and consequently no stereochemical assignments could be made in ring A. If its stereochemistry could be established, it would also apply to barringtogenic acid (VII),[20] barringtogenol (VIII)[20] and terminolic acid (X)[21] since these three triterpenes have been related to arjunolic acid (VI) in a stereochemically unambiguous manner.

Arjunolic acid (VI) and medicagenic acid (Ia) cannot have the identical configuration at C-2, C-3 and C-4 since the tetrol IX, obtained by lithium aluminum hydride treatment of diacetoxymedicagenic acid (Ia), is not identical with barringtogenol (VIII), the common lithium aluminum hydride reduction product of arjunolic acid (VI) and barringtogenic acid (VII). In fact, when the lead tetraacetate oxidation[19] of arjunolic acid (VI) was carried out quantitatively,[13] a value of $k = 1.07 \times 10^{-3}$ l.-mole^{-1} sec.$^{-1}$ was obtained, which is only about one-thirtieth of that observed with medicagenic acid (Ia) but in good agreement with the reported values[12b,13] (1.63 to 1.85×10^{-3}) for steroidal 2α,3β-diols. Consequently, we assign[14] the 2α,3β-orientation to the glycol grouping of arjunolic acid (VI) and *ipso facto* of all the other

(12) (a) J. Pataki, G. Rosenkranz and C. Djerassi, THIS JOURNAL, **73**, 5375 (1951); (b) C. Djerassi, L. B. High, T. T. Grossnickle, R. Ehrlich, J. A. Moore and R. B. Scott, *Chemistry & Industry*, 474 (1955); (c) C. Djerassi, T. T. Grossnickle and L. B. High, THIS JOURNAL, **78**, 3166 (1956); (d) H. L. Slates and N. L. Wendler, *ibid.*, **78**, 3749 (1956).

(13) C. Djerassi and R. Ehrlich, *J. Org. Chem.*, **19**, 1351 (1954); for corrected value of 2β,3β-isomer, see footnote 58 in ref. 12c.

(14) It seems that tbe additional substituents at C-4 play no role since the agreement in the lead tetraacetate oxidation in the arjunolic acid and asiatic acid series and the steroidal 2α,3β-diol is also excellent.

(15) *Cf.* L. Ruzicka, G. Müller and H. Schellenberg, *Helv. Chim. Acta*, **22**, 767 (1939); D. H. R. Barton and C. J. W. Brooks, *J. Chem. Soc.*, 257 (1951).

(16) *Cf.* C. Djerassi and A. W. Lippman, THIS JOURNAL, **77**, 1825 (1955); C. Djerassi and H. G. Monsimer, *ibid.*, **79**, 2901 (1957).

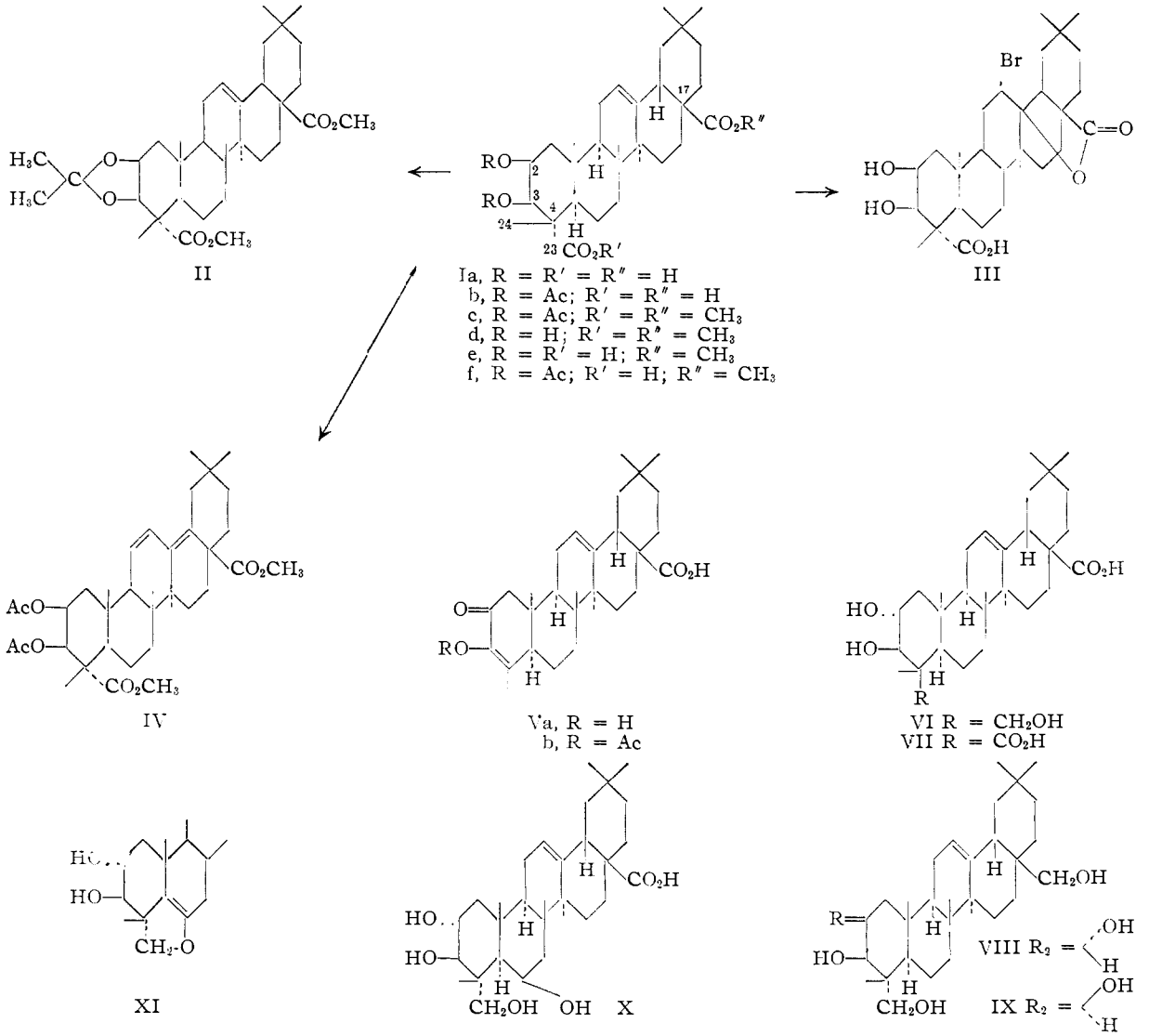
(17) The axial 24 position is excluded since such methyl esters (*e.g.*, β-boswellic acid) can be saponified only with great difficulty (P. Bilham, G. A. R. Kon and W. C. J. Ross, *J. Chem. Soc.*, 35 (1942); A. Vogel, O. Jeger and L. Ruzicka, *Helv. Chim. Acta*, **34**, 2321 (1951)).

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

(19) F. E. King, T. J. King and J. M. Ross, *J. Chem. Soc.*, 3995 (1954). We are greatly indebted to Prof. King for a generous supply of arjunolic acid which permitted the experimental completion of our work.

(20) R. Anantaraman and K. S. Madhavan Pillai, *J. Chem. Soc.* 4369 (1956).

(21) F. E. King and T. J. King, *ibid.*, 4469 (1956).



triterpenes (VII, VIII, X) which have been related to it. Furthermore, asiatic acid (XVII),[22] an isomer of arjunolic acid belonging to the α-amyrin series, is also oxidized at substantially the same rate ($k = 1.60 \times 10^{-3}$ l. - mole^{-1} sec.$^{-1}$) by lead tetraacetate, thus indicating the presence of a $2\alpha,3\beta$-glycol moiety (see XVII) in this triterpene as well. It is interesting to note that the naturally-occurring steroidal sapogenins also possess either the 2α, 3β-[12] or $2\beta,3\beta$-orientations,[23] while no $2\alpha,3\alpha$- and $2\beta,3\alpha$-diols have so far been observed in nature.

The only unknown stereochemical feature of these four triterpenes of the arjunolic acid type is the orientation of the C-4 substituent. The fact that these substances form 1,3-acetonides[24] has no stereochemical significance because acetonide formation should be equally feasible[11] with a $3\beta,23$- as well as a $3\beta,24$-diol. On the other hand, King and King[21] in their elegant structure proof of terminolic acid (X) have shown that various anhydroterminonic acid derivatives must possess partial structure XI (without the stereochemical assignment). Inspection of models indicates that such cyclic enol ether formation seems only feasible with an equatorial substituent at C-4. Since terminolic acid (X) has been converted[21] into arjunolic acid (VI), the above cited stereochemical evidence for the glycol grouping coupled with the steric implications of enol ether formation (XI) leads to the complete stereoformulas VI, VII, VIII and X for arjunolic acid, barringtogenic acid, barringtogenol and terminolic acid.[25]

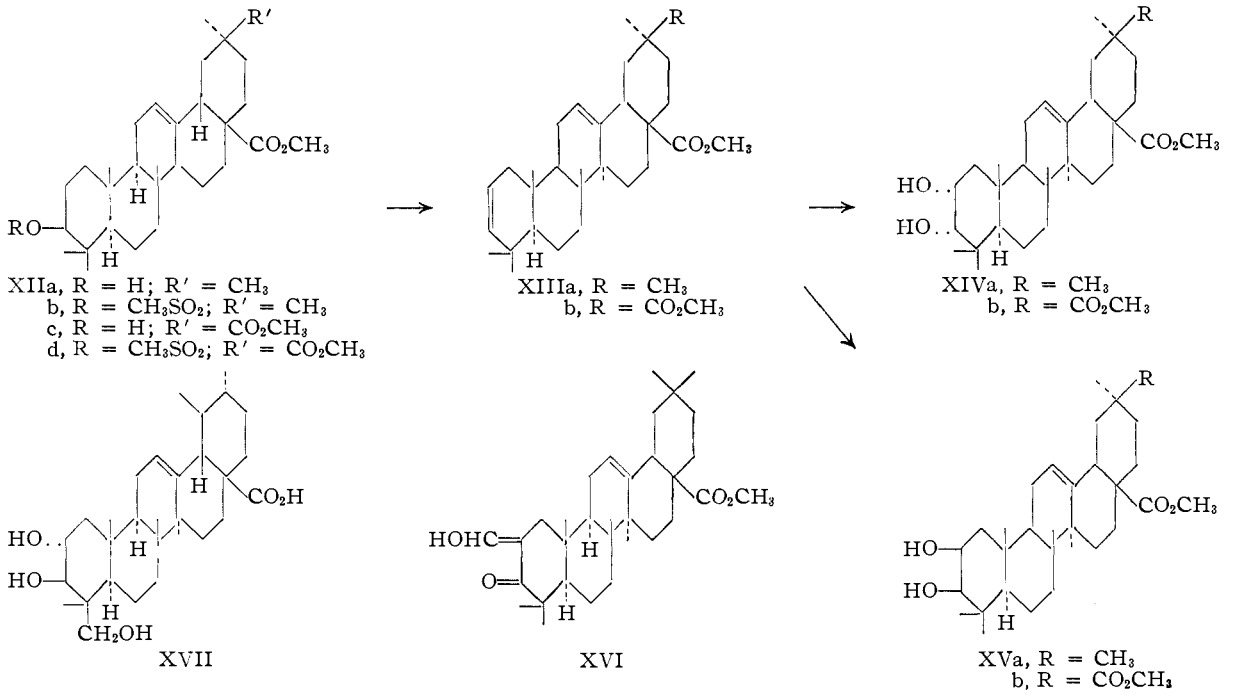
Prior to the definite location of the second carboxyl group of medicagenic acid (Ia), certain model experiments toward the synthesis of ring A glycols were undertaken and these are outlined briefly below. The reactions were carried out both in the methyl oleanolate series (XIIa) as well as with the corresponding 30 carboxylic acid derivative XIIc,

(22) P. Boiteau, A. Buzas, E. Lederer and J. Polonsky, *Nature*, **163**, 258 (1949); J. Polonsky, *Bull. soc. chim. France*, 173 (1953). We are greatly indebted to Dr. J. Polonsky for a generous gift of asiatic acid.

(23) C. Djerassi and J. Fishman, THIS JOURNAL, **77**, 4291 (1955).

(24) This was proved definitely in the terminolic acid series (ref. 21) and hence should also apply to the other members of that group

(25) The axial (β) orientation of the hydroxyl group at C-6 has already been established earlier (ref. 21).



which can be prepared from queretaroic acid.[26] In each instance, the appropriate 3-mesylate was boiled with pyridine[27] or with sodium iodide in methyl ethyl ketone solution and the resulting olefin XIII was hydroxylated with osmium tetroxide. Decomposition of the osmate ester with hydrogen sulfide[28] and careful chromatography yielded in each case two glycols. It appears, therefore, that frontal attack is also feasible in spite of the additional C-4 methyl group.

As a possible route to triterpenoid ring A diosphenols, the ozonolysis of 2-hydroxymethylene methyl oleanonate (XVI) was attempted,[29] but the yield of desired product was so poor as to discourage further work along those lines.

Experimental[30]

Improved Isolation of Medicagenic Acid.—The crude saponin[5] was obtained from a hot, aqueous extract of dehydrated alfalfa (*Medicago sativa*) meal in the form of a cholesterol addition product and then regenerated by partition between water, ethanol and benzene (0.5:1:1). A 200-g. portion of saponin was heated under reflux for 72 hr. with 12 l. of 1 *N* ethanolic hydrochloric acid (1:1), diluted with water, and the sapogenin was filtered, washed and dried at 100°. Purification was best accomplished *via* the diacetate (pyridine–acetic anhydride at room temperature), which was chromatographed on 37 times its weight of magnesia–silica gel (Floridin Co., Tallahassee, Fla.). Elution with benzene–methanol (92:8) and recrystallization from methanol afforded a new[5] analytical sample of medicagenic acid diacetate (Ib), m.p. 210–212°, $[\alpha]D$ +94°.

Anal. Calcd. for $C_{34}H_{50}O_8$: C, 69.59; H, 8.59; acetyl, 14.60. Found: C, 69.60; H, 8.53; acetyl, 14.60.

The free acid Ia, obtained by saponification of the chromatographed diacetate, exhibited the same constants as published earlier.[5]

Diacetoxy Dimethyl Medicagenate (Ic).—The above diacetate was methylated in methanol solution with ethereal diazomethane, and the resulting product was chromatographed on Alcoa alumina (Grade F-20) deactivated with 3% of 10% acetic acid and eluted with benzene–ether (4:1). Crystallization from methanol gave colorless rhombs, m.p. 235–238°, $[\alpha]D$ +87°; $\lambda_{max}^{CHCl_3}$ 5.7–5.76 (broad band) and 8.0 μ; λ_{max}^{Nujol} 5.68, 5.77 and 8.03 μ with inflection at 5.64 μ.

Anal. Calcd. for $C_{36}H_{54}O_8$: C, 70.33; H, 8.85; O, 20.82. Found: C, 70.06; H, 8.65; O, 20.82.

Dimethyl Medicagenate (Id).—A solution of 200 mg. of the diacetoxy dimethyl ester Ic in 75 cc. of methanol and 8 cc. of 50% aqueous dioxane was boiled for 40 min. with 380 mg. of potassium carbonate, approximately one-half of the solvent being removed during that time. Dilution with water, extraction with ether, chromatography on 12 g. of deactivated (3% of 10% acetic acid) alumina and elution with ether–methanol (19:1) afforded 140 mg. of the dimethyl ester, m.p. 221–225°. Saponification could also be accomplished by heating under reflux for 3 hr. with 5% methanolic potassium hydroxide solution. The analytical sample crystallized from methanol as colorless rods, m.p. 221.5–224°, $[\alpha]D$ +93.5°; $\lambda_{max}^{CHCl_3}$ 2.8–2.9 (broad), 5.78–5.82 μ; λ_{max}^{Nujol} 2.82, 2.92, 5.76 and 5.91 μ (very well resolved).[9] The separation of the ester bands in XIVb and XVb (see below) proceeds in the opposite direction toward shorter wave length.

Anal. Calcd. for $C_{32}H_{50}O_6$: C, 72.41; H, 9.50; O, 18.09; methoxyl, 11.70. Found: C, 72.10; H, 9.57; O, 18.29; methoxyl, 11.51.

Partial Saponification of Diacetoxy Dimethyl Medicagenate (Ic).—A solution of 650 mg. of the diacetoxy dimethyl ester (Ic) in 62 cc. of 10% methanolic potassium hydroxide was heated under reflux for 18 hr.[31] After dilution with water and ether extraction (discarded), the aqueous phase was acidified and the crude monomethyl ester Ie isolated

(26) C. Djerassi, J. A. Henry, A. J. Lemin, T. Rios and G. H. Thomas, THIS JOURNAL, **78**, 3783 (1956).

(27) *Cf.* C. R. Noller and P. J. Hearst, *ibid.*, **72**, 625 (1950); A. B. Burns, A. R. H. Cole, B. J. Parkes and D. E. White, *Austral. J. Chem.*, **9**, 406 (1956).

(28) D. H. R. Barton and D. Elad, *J. Chem. Soc.*, 2090 (1956).

(29) *Cf.* C. Djerassi, R. Riniker and B. Riniker, THIS JOURNAL, **78**, 6362 (1956), for successful ozonolysis of *trans*-9-methyl-2-hydroxymethylenedecalone-1.

(30) All melting points were determined on the Kofler block. Unless noted otherwise, rotations were measured in 1-dcm. tubes in chloroform solution. We are indebted to Mrs. Dolores Phillips for all spectral measurements and to Dr. A. Bernbardt (Mülheim, Germany) for the microanalyses.

(31) It has been reported (ref. 20) that the dimethyl ester of barringtogenic acid (VII) is not hydrolyzed by boiling for 4 hr. with 10% ethanolic potassium hydroxide, but this may have been too short a period of time.

with ether. Acetylation of the total material with acetic anhydride–pyridine at room temperature and recrystallization from methanol gave 260 mg. of plates of the **diacetoxy monomethyl ester If**, m.p. 257–259° (losing transparency at 160–170°), $[\alpha]D$ +76°, $\lambda_{max}^{CHCl_3}$ 5.7 (broad band) and inflection at 5.80 μ.

Anal. Calcd. for $C_{35}H_{52}O_8 \cdot H_2O$: C, 67.93; H, 8.80; O, 23.27; methoxyl, 5.02. Found: C, 67.83; H, 8.87; O, 23.07; methoxyl, 5.61.

A sample of the above diacetate Ie was heated under reflux for 3 hr. with 10% methanolic potassium hydroxide solution, but the resulting **monomethyl ester Ie** was obtained as a gel after repeated attempts at crystallization; $[\alpha]D$ +98° (abs. ethanol).

Anal. Calcd. for $C_{31}H_{48}O_6$: neut. equiv., 517; methoxyl, 6.00. Found: neut. equiv., 515; methoxyl, 5.81.

No bromo lactone was produced (no infrared lactone band in crude product) in chloroform solution. On the other hand, the amorphous bromo lactone III[5] derived from medicagenic acid exhibited infrared bands (Nujol) at 5.60 and 5.83 μ.

Dimethyl Medicagenate Acetonide (II).—A mixture of 100 mg. of dimethyl medicagenate (Id) in 10 cc. of acetone and 0.1 cc. of concd. sulfuric acid[11] was left at room temperature for 3 days, sodium carbonate was added and the product was isolated with ether. Chromatography of the crude product on deactivated (3% of 10% acetic acid) alumina furnished most of the product in the benzene eluates (note: dimethyl medicagenate (Id) itself requires ether–methanol for elution on identical alumina), but the acetonide could not be crystallized and the amorphous product ($\lambda_{max}^{CS_2}$ 5.75 μ (shoulder at 5.71 μ) but no hydroxyl absorption) was analyzed.

Anal. Calcd. for $C_{35}H_{54}O_6$: C, 73.64; H, 9.54; O, 16.82. Found: C, 73.28; H, 9.64; O, 16.87.

Oxidation of Diacetoxy Dimethyl Medicagenate (Ic) with Selenium Dioxide.—The diacetoxy dimethyl ester Ic (93 mg.) was heated under reflux for 2 hr. with 93 mg. of selenium dioxide and 20 cc. of glacial acetic acid. Filtration of the solid, washing with benzene and evaporation left a gum which was passed in ether solution through 8 g. of deactivated alumina. The eluted gum (72 mg.) exhibited λ_{max}^{EtOH} 243, 251 and 260 mμ, log ϵ 4.17, 4.19 and 4.15. The spectral data are fully consistent with the $\Delta^{11,13(18)}$-diene[15] formulation IV, but the product could not be crystallized even after repeated chromatography.

Quantitative Lead Tetraacetate Oxidations.—The oxidations were carried out in acetic acid solution at room temperature exactly as described for the corresponding steroidal glycols.[13] The oxidation of dimethyl medicagenate (Id) was complete in *ca.* 25 min. ($k = 31 \times 10^{-3}$ l.-mole^{-1} sec.$^{-1}$) while the oxidation of arjunolic acid (VI) required *ca.* 12–14 hr. (1.07×10^{-3} l.-mole^{-1} sec.$^{-1}$) and that of asiatic acid[22] (XVII) about 10 hr. ($k = 1.60 \times 10^{-3}$ l.-mole^{-1} sec.$^{-1}$).

Δ^{12}-Oleanene-2β,3β,23,28-tetrol (IX).—A 200-mg. sample of medicagenic acid diacetate (Ib) was heated under reflux overnight with excess lithium aluminum hydride in ether solution. After addition of ethyl acetate followed by dilute sulfuric acid, the tetrol was extracted with ether and recrystallized from acetone; needles, m.p. 257–259°, $[\alpha]D$ +95°, no infrared carbonyl absorption. This tetrol differed completely from the isomeric tetrol VIII obtained on lithium aluminum hydride reduction of arjunolic acid (VI).

Anal. Calcd. for $C_{30}H_{50}O_4$: C, 75.90; H, 10.62; O, 13.48. Found: C, 75.37; H, 10.41; O, 14.08.

$\Delta^{3,12}$-23-Nor-oleadien-2-one-3-ol-28-oic Acid (Va). (a) From Medicagenic Acid (Ia).—A 200-mg. sample of the acid dissolved in 50 cc. of acetone was treated at 5–10° over a period of 25 minutes with a standard chromium trioxide solution (2.67 g. of chromium trioxide, 7.7 cc. of water and 2.3 cc. of concd. sulfuric acid) until a slight excess was present (*ca.* 0.3 cc. required). Addition of water, extraction with ether and removal of the solvent afforded a gum ($[\alpha]D$ +100°, λ_{max}^{Nujol} 5.78 and 5.88 μ) which could not be crystallized from methanol. It was heated under reflux for 1 hr. with 10 cc. of 10% methanolic potassium hydroxide containing a few drops of water to dissolve the potassium salt. The mixture was diluted with water, acidified, extracted with ether and the ether residue was dried by azeotropic distillation with benzene. Crystallization from methanol gave 125 mg. of plates, m.p. 168–172°, $[\alpha]D$ +145°; λ_{max}^{Nujol} 2.93, 3.08, 5.80, 5.98 and 6.05 μ; λ_{max}^{EtOH} 281 mμ, log ϵ 4.02; $\lambda_{max}^{KOH\text{-}EtOH}$ 329 mμ, log ϵ 3.30; dark green color with ferric chloride.

Anal. Calcd. for $C_{29}H_{42}O_4$: C, 76.61; H, 9.31; O, 14.08. Found: C, 76.28; H, 9.50; O, 14.69.[32]

The crystalline diosphenol (Va) (200 mg.) was acetylated at room temperature overnight with acetic anhydride and pyridine. Working up in the usual way gave a solid which was chromatographed on 8.8 g. of Florisil. Elution with benzene–ether (7:3 and 1:1) gave 110 mg. of the **enol acetate Vb** which crystallized as needles from acetone–hexane and from methanol; m.p. 254–259° dec., $[\alpha]D$ +162°; λ_{max}^{Nujol} 5.62, 5.91, 6.04 and 8.20 μ; λ_{max}^{EtOH} 247 mμ, log ϵ 3.99.

Anal. Calcd. for $C_{31}H_{44}O_5 \cdot CH_3OH$: C, 72.69; H, 9.15. Found: C, 72.61; H, 8.67.

(b) **From Arjunolic Acid (VI).**—The oxidation of 250 mg. of arjunolic acid (VI)[19] was carried out exactly as described above and gave 110 mg. of the diosphenol, m.p. 170–175°, undepressed when mixed with the sample prepared according to (a), $[\alpha]D$ +149°, λ_{max}^{EtOH} 281 mμ, log ϵ 4.07, $\lambda_{max}^{KOH\text{-}EtOH}$ 329 mμ, log ϵ 3.33, green color with ferric chloride. The infrared absorption spectrum was identical with that of the specimen obtained from medicagenic acid.

Anal. Found: C, 76.05; H, 9.52; O, 14.16.

The enol acetate was prepared in the usual fashion and exhibited m.p. 257–260° dec., $[\alpha]D$ +156°, λ_{max}^{EtOH} 247 mμ, log ϵ 4.06. Identity with the above described sample was demonstrated by infrared comparison and undepressed mixture melting point.

Anal. Found: C, 72.76; H, 8.89.

Methyl Oleanolate 3-Mesylate (XIIb).[33]—A mixture of 1.0 g. each of methyl oleanolate (XIIa) and methanesulfonyl chloride in 20 cc. of dry pyridine was left at room temperature for 6 hr., poured onto ice and the crystals collected. Recrystallization from methanol afforded 850 mg. of the mesylate, m.p. 121–123°, which was suitable for the next step. The analytical sample exhibited m.p. 125–126°, $[\alpha]D$ +64°.

Anal. Calcd. for $C_{32}H_{52}O_5S$: C, 70.04; H, 9.55. Found: C, 70.55; H, 9.85.

Methyl $\Delta^{9,12}$-Oleadien-28-oate (XIIIa).—A solution of 250 mg. of the mesylate XIIb in 25 cc. of pyridine was heated under reflux for 22 hr., and most of the solvent was removed *in vacuo*. Extraction with ether, washing, drying, evaporation and recrystallization from methanol yielded 130 mg. of the olefin, m.p. 180–184°, $[\alpha]D$ +101°.

Alternatively, the mesylate (235 mg.) was heated under reflux for 25 hr. with 800 mg. of sodium iodide and 25 cc. of methyl ethyl ketone. The resulting product had to be chromatographed and recrystallization of the benzene eluates from methanol produced 70 mg. of the olefin with m.p. 176–180°.

Anal. Calcd. for $C_{31}H_{48}O_2$: C, 82.24; H, 10.69; O, 7.07. Found: C, 81.88; H, 10.65; O, 7.39.

That no rearrangement had occurred was demonstrated by catalytic hydrogenation with platinum oxide in ethyl acetate solution which furnished methyl 3-desoxyoleanolate, m.p. 167–169°, $[\alpha]D$ +80°, identified in the usual manner with an authentic specimen.[26]

Dimethyl Δ^{12}-Oleanen-3β-ol-28,30-Dioate (XIIc).—To a solution of 800 mg. of methyl Δ^{12}-oleanen-3-one-28,30-dioate[26] in 120 cc. of methanol was added 2.8 g. of sodium borohydride dissolved in 55 cc. of methanol and 15 cc. of water. After cooling to room temperature, the mixture was permitted to stand for 80 minutes, diluted with much water containing some hydrochloric acid and the solid was filtered. Recrystallization from methanol led to 740 mg. of plates, m.p. 234–236°, $[\alpha]D$ +92°.

Anal. Calcd. for $C_{32}H_{50}O_5$: C, 74.67; H, 9.79; O, 15.54. Found: C, 74.86; H, 9.75; O, 15.34.

The above alcohol (700 mg.) was converted in the usual manner into the **mesylate XIId** (680 mg.), m.p. 150–151° (from methanol), $[\alpha]D$ +71°.

(32) Neutralization equivalent determinations (0.1 *N* NaOH) gave ambiguous values (found: 373) since the diosphenol was apparently also titrated in part.

(33) This compound was prepared by Dr. F. W. Donovan.

Anal. Calcd. for $C_{33}H_{52}O_7S$: C, 66.86; H, 8.84; O, 18.92. Found: C, 66.21; H, 8.50; O, 18.90.

Dimethyl $\Delta^{2,12}$-Oleadiene-28,30-dioate (XIIIb).—The mesylate XIId (600 mg.) was heated under reflux with pyridine for 19 hr., and the product was isolated in the usual way including chromatography and elution with benzene. Recrystallization from methanol gave 255 mg. of colorless plates of the olefin, m.p. 192–195°, $[\alpha]D$ +134°.

Anal. Calcd. for $C_{32}H_{48}O_4$: C, 77.37; H, 9.74. Found: C, 76.88; H, 9.33.

Hydroxylation of Methyl $\Delta^{2,12}$-Oleadien-28-oate (XIIIa).—The methyl ester XIIIa (390 mg.) in 16 cc. of dioxane was left at room temperature for 8 days with 700 mg. of osmium tetroxide. The osmate ester was decomposed by passing hydrogen sulfide[28] into the solution and filtering the insoluble sulfide. The solvent was removed, and the residue was chromatographed carefully on 20 g. of alumina deactivated with 5% of 10% acetic acid. After removing 80 mg. of unreacted starting material with benzene, the benzene–ether (3:1 and 1:1) eluates afforded 147 mg. of solid, presumably methyl Δ^{12}-**oleanene-2α,3α-diol-28-oate (XIVa)**, which crystallized as needles from methanol–chloroform; m.p. 258–262°, $[\alpha]D$ +85°.

Anal. Calcd. for $C_{31}H_{50}O_4$: C, 76.50; H, 10.36; O, 13.15. Found: C, 75.96; H, 10.30; O, 13.82.

Further elution with 1:1 benzene–ether gave 28 mg. of the **2β,3β-isomer XVa**, which crystallized as plates from methanol–chloroform, m.p. 276–284° (depressed to 245–253° on admixture with XIVa), $[\alpha]D$ +72°.

Anal. Found: C, 75.85; H, 10.00.

Hydroxylation of Dimethyl $\Delta^{2,12}$-Oleadiene-28,30-dioate (XIIIb).—The reaction was carried out exactly as described above for XIIIa except that 450 mg. of dimethyl ester and 1.0 g. of osmium tetroxide was used. Benzene removed 190 mg. of unreacted olefin and the diols were eluted with ether. The initial material (37 mg., m.p. 227–229°, $[\alpha]D$ +112°) was followed by 70 mg. of an intermediate fraction (m.p. 248–260°) and finally by 60 mg. of a second diol XVb, m.p. 274.5–276°, $[\alpha]D$ +95°.

Anal. Calcd. for $C_{32}H_{50}O_6$: C, 72.41; H, 9.50; O, 18.09. Found: C, 72.18; H, 9.40; O, 17.92.

Both diols showed resolution of the ester bands in Nujol mull to the same extent (5.72 and 5.77 μ) which differed very considerably from the shift observed with dimethyl medicagenate (Id). A 20-mg. sample of the mixed diols was oxidized in the manner described above for medicagenic acid (Ia) and the oxidation product was treated with base. The oil could not be crystallized but the position of its ultraviolet absorption maximum (λ_{max}^{EtOH} 266 mμ, $\lambda_{max}^{KOH-EtOH}$ 306 mμ) is in good agreement with expectation since this diosphenol contains one less substituent on the double bond as compared with Va.

Methyl 2-Hydroxymethyleneoleanonate (XVI).—Dry sodium methoxide (from 200 mg. of sodium), 15 cc. of ether and 1.8 cc. of dried and freshly distilled ethyl formate were stirred for 30 min. in an atmosphere of nitrogen followed by addition of 1.45 g. of methyl oleanonate in 30 cc. of benzene. After stirring under nitrogen for 17 hr., dilute hydrochloric acid was added, the organic layer was separated and washed well with water and dried. Recrystallization from methanol–chloroform afforded 1.2 g. of plates, m.p. 205–207.5°, $[\alpha]D$ +109°, dark brown color with ferric chloride.

Anal. Calcd. for $C_{32}H_{48}O_4$: C, 77.37; H, 9.74; O, 12.88. Found: C, 76.69; H, 9.79; O, 13.10.

A 300-mg. sample was ozonized in methylene chloride solution at −70° for 5 minutes in the earlier described fashion[29] and the ozonide was decomposed with ferrous sulfate. Separation into acidic (120 mg.) and neutral (148 mg., brown color with ferric chloride) fractions followed by warming of the latter with alkali and chromatography on deactivated alumina led to only 15 mg. of oil. Its ultraviolet absorption spectrum (λ_{max}^{EtOH} 270 mμ) indicated that it probably contained some of the desired diosphenol, but this approach was not pursued due to the poor yield. Ozonolysis in ethyl acetate solution at room temperature produced material which on the basis of its infrared spectrum seemed to be largely anhydride.

DETROIT, MICHIGAN
ALBANY, CALIFORNIA

[CONTRIBUTION FROM THE MERCK SHARP & DOHME RESEARCH LABORATORIES, DIVISION OF MERCK & CO., INC.]

Identification and Synthesis of 4,17-Pregnadien-20-ol-21-al-3,11-dione Acetate[1]

BY R. E. BEYLER AND FRANCES HOFFMAN

RECEIVED APRIL 19, 1957

The synthesis and reactions of 4,17-pregnadien-20-ol-21-al-3,11-dione acetate and 4,17-pregnadiene-11β,20-diol-21-al-3-one 20-acetate are described.

In the course of an investigation of steroids related to adrenocortical hormones, we encountered a compound of unusual chemical and biological properties. This material was isolated in very small yield from mother liquors obtained in the synthesis of cortisone *via* the 3-dinitrophenylhydrazone. The most distinctive property it possessed was its high extinction coefficient in the ultraviolet, λ_{max}^{MeOH} 241 mμ, $E\%$ 617. The source of the compound,[2] together with the ultraviolet absorption and elemental analysis, suggested that it might be 4,16-pregnadien-21-ol-3,11,20-trione acetate, which we desired for further synthetic studies. That this was not the correct structure was conclusively shown when an authentic sample of this compound was made available to us.[3] The ultraviolet spectra of the two compounds are similar but all other properties are different.[4]

Alternative structures for steroids with two chromophoric groups (presumed to be conjugated carbonyl groups) are not plentiful. The 20-enol acetate-21-aldehyde[5] structure (VIa) was a logical candidate, particularly in view of the following properties which the compound possessed: 5.70 (Nujol) or 5.68 ($CHCl_3$) μ band in the infrared typical of an enol acetate; Porter–Silber test very rapid (similar to "cortisone aldehyde"); instability on acid-washed alumina; positive blue tetrazolium test (hereafter abbreviated "BT test"). A negative Schiff test (other 21-aldehydes are positive) was somewhat disturbing, however.

(1) Presented in part at the Ninth Annual Meeting-in-Miniature of the North Jersey Section at Seton Hall University, South Orange, N. J., on January 28, 1957.

(2) H. Reich and B. K. Samuels, *J. Org. Chem.*, **19**, 1041 (1954), have obtained Δ^{16}-steroids from 17-hydroxy-20-dinitrophenylhydrazones by treatment with acid.

(3) We are grateful to Dr. W. F. McGuckin for providing us with a sample and physical constants prior to the publication.[4]

(4) W. F. McGuckin and H. L. Mason, THIS JOURNAL, **77**, 1822 (1955).

(5) G. A. Fleisher and E. C. Kendall, *J. Org. Chem.*, **16**, 573 (1951), have reported a 20-enol acetate 21-aldehyde in another series.