Isolation of 19α-H-Lupeol from *Maclura pomifera*

J. E. GEARIEN ˣ **and MICHAEL KLEIN**

Abstract □ A reinvestigation of the constituents of the Osage orange (*Maclura pomifera*) yielded, in addition to the previously reported triterpenes (lupeol, butyrospermol, and lupane-3β,20-diol), the pigments osajin and pomiferin, and a previously unreported constituent. The structure of this new compound was investigated. On the basis of spectroscopic and chemical data, it appeared to be an epimer of lupeol and is referred to as 19α-H-lupeol.

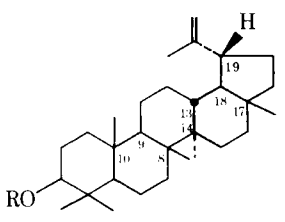
Keyphrases □ Osage orange *(Maclura pomifera)*—reinvestigation of constituents, isolation and identification of 19α-H-lupeol □ *Maclura pomifera* (Osage orange)—reinvestigation of constituents, isolation and identification of 19α-H-lupeol □ 19α-H-Lupeol—isolation, identification from Osage orange *(Maclura pomifera)*

The resistance of the Osage orange to insect attack led to a reexamination of the constituents of its fruit. Along with previously reported constituents (1–5), an unreported epimer of lupeol was also isolated.

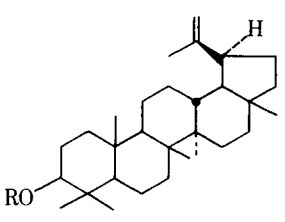
The dried fruit of the Osage orange yielded a mixture of triterpenes and pigments when extracted with petroleum ether. After separation of the pigments by chromatography, the triterpene constituents were obtained as a viscous oil. After refluxing the oil with alcoholic potassium hydroxide and extraction with ether, a solid containing the triterpene alcohols was obtained. When chromatographed on basic alumina with various solvents (Scheme I), a partial separation of the constituents was obtained.

Each fraction was treated with a mixture of acetic anhydride–pyridine. Butyrospermyl acetate and lupenyl acetate (I*b*) were isolated from the petroleum ether fraction; lupane-3β,20-diol 3-monoacetate was isolated from the alcohol fraction when eluted with benzene–ethanol (3:1). In addition, elution of a fraction with petroleum ether–benzene (4:1) from basic alumina gave a compound later shown to be 19α-H-lupenyl acetate (II*b*), a previously unreported constituent whose IR spectrum was similar to that of I*b*.

Their NMR spectra appeared identical except in the methine region at δ 1.59–1.65. Mass spectrum fragmentation patterns, of which several peaks were assigned previously (4) as being characteristic of the lupane ring system, were identical except for the percent relative abundance for certain peaks and therefore did not provide conclusive data in distinguishing between the two. Parent ions were *m/e* 468 for I*b* and II*b* and *m/e* 426 for the alcohols I*a* and II*a*. Specific rotations differed, and mixed melting points were depressed.



I*a*: R = H
I*b*: R = —$COCH_3$



II*a*: R = H
II*b*: R = —$COCH_3$

EXPERIMENTAL[1]

Materials—The ripe fruit[2] of the Osage orange tree was gathered in a grove in Chicago, Ill., in fall 1967. The alumina[3] was obtained commercially. Chromatographic solvents were distilled prior to use. Petroleum ether used for chromatography had a boiling range of 40–60°.

Chromatographic columns were prepared by mixing the adsorbent with petroleum ether into a slurry, pouring into a column, and allowing to settle. Excess petroleum ether was recycled through the column. Compounds were then adsorbed onto the column and eluted with the indicated solvents.

Extraction of Triterpenes and Pigments—The ripe fruit of the Osage orange tree was dried in an oven at 60°, and it was then broken with a hammer into fine pieces and crushed to a powder in a mortar and pestle. It (74 g) was extracted with petroleum ether in a soxhlet extraction apparatus for 48 hr. After this time, the extraction material was replaced with a fresh portion.

This procedure was repeated 50 times until a total of 3.5 kg of dried fruit was extracted. Evaporation of the extract on the flash evaporator yielded a mixture of a solid yellow powder in a brown-colored oil (600 g, which amounted to 17.1% of the dried fruit).

Separation of Triterpenes and Pigments—The petroleum ether extract (70 g) was placed on a basic alumina column (1.5 kg) and eluted with 500 ml each of petroleum ether, carbon tetrachloride–petroleum ether (1:1), and finally carbon tetrachloride. The remaining yellow material on the column was eluted exhaustively with 500 ml of methanol. Evaporation of the solvent yielded a dark-brown oil (15 g), from which the pigments osajin and pomiferin were obtained, following the procedure of Wolfram *et al.* (5).

Hydrolysis of Osage Orange Extract—The fractions of Osage orange extract that were eluted with petroleum ether, petroleum ether–carbon tetrachloride (1:1), and carbon tetrachloride were combined. The solvent was evaporated on the flash evaporator, yielding a thick brown oil (50 g). To this oil was added 5 liters of 95% ethanol and 20.0 g of potassium hydroxide. The resulting mixture was heated at reflux for 72 hr.

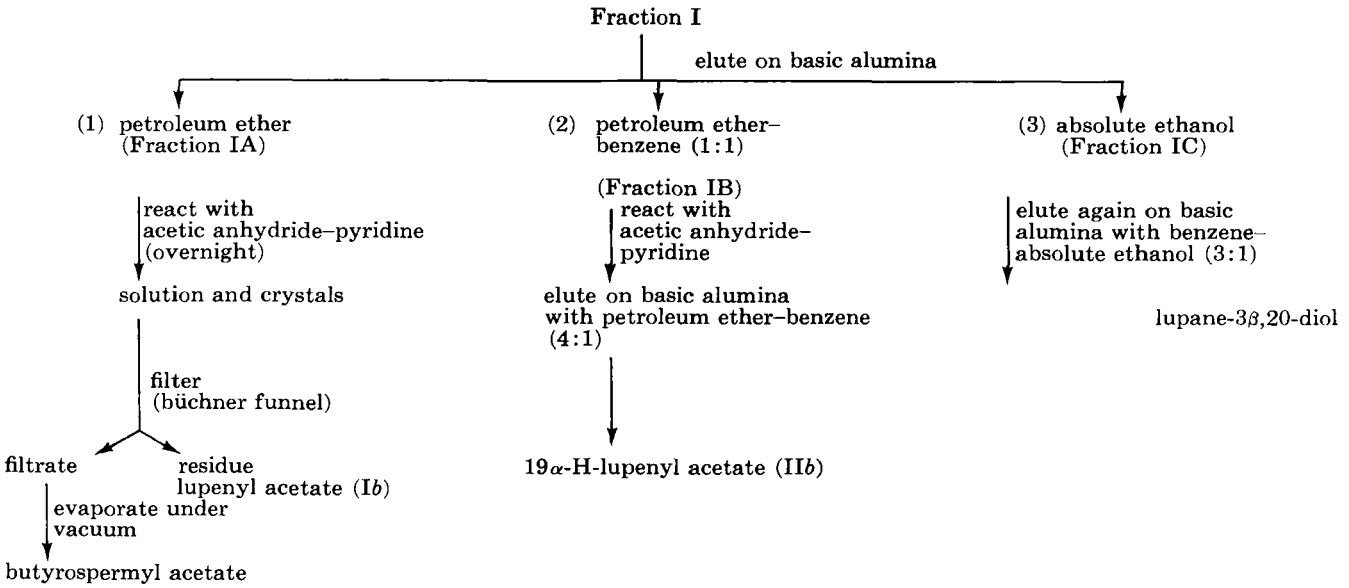
The alcohol was removed on a flash evaporator, leaving a thick yellow oil. Water (1 liter) was added, and the mixture was extracted with three 1-liter portions of methylene chloride. The methylene chloride portion was evaporated, yielding 25 g of light-brown solid material.

Separation of Triterpene Alcohols—The mixture of triterpene alcohols (25 g) was chromatographed on 200 g of basic alumina. Exhaustive elution with petroleum ether (1.5 liters) yielded a

[1] Melting points were determined on a Thomas-Hoover capillary melting-point apparatus and are uncorrected. Elemental analyses for carbon and hydrogen were performed by Dr. Kurt Eder, Geneva, Switzerland, and by Micro-Tech Laboratories, Inc., Skokie, Ill. The NMR spectra were determined using a Bruker model HFXO5 90-MHz spectrometer and were recorded downfield from tetramethylsilane as an internal reference. IR spectra were obtained with a Perkin-Elmer recording spectrophotometer, model 337, using KBr pellets. IR spectra with microquantities were determined with the aid of a Wilks scientific model 9 single-beam internal reflection attachment on a KRS-5 reflector plate (50 × 20 mm). Mass spectral data were obtained by means of the Hitachi Perkin-Elmer model RMU-6D spectrometer. UV spectra were obtained using a Perkin-Elmer model 202 double-beam recording spectrophotometer. Optical rotation were performed on a polarometer (No. 283) by O. C. Rudolph & Sons, Caldwell, N.J., in chloroform. Authentic samples of lupeol acetate, butyrospermol, and lupane-3β,20-diol 3-monoacetate were obtained through the courtesy of Professor K. G. Lewis, University of Australia, Armisdale, WSW.

[2] The fruit was identified by Professor Frank A. Crane, Department of Pharmacognosy and Pharmacology, University of Illinois at the Medical Center, Chicago. A sample is kept at that institution.

[3] Chromatographic Alumina F-20 (Activity I, basic), Alcoa Chemicals.



Scheme I—Separation of constituents

light-brown solution which, after removal of the solvent, gave a solid (Fraction I, 15 g). Further purification of Fraction I (2.5 g) by chromatography was required, eluting on basic alumina (100 g). Three solvent systems were applied. The fraction eluted with petroleum ether was labeled IA (1.1 g). Elution with petroleum ether–benzene (1:1) yielded Fraction IB (0.25 g), and final elution with absolute ethanol yielded Fraction IC (0.1 g).

Fraction IA: A Mixture of Butyrospermol and Lupeol—Removal of the solvent of Fraction IA yielded a brown solid. Fraction IA was recrystallized from acetone, collected on a büchner funnel, and washed with acetone to remove the adhering tar-like material. The fine white crystals remaining were allowed to air dry and then were recrystallized from 95% ethanol to give a white solid (1.0 g), which was treated overnight at room temperature with pyridine (10 ml) and acetic anhydride (15 ml) to give a solid monoacetate. The reaction mixture was filtered and the solid was recrystallized three times from 95% ethanol to give 0.48 g of lupenyl acetate, mp 213–214°, $[\alpha]_D^{25}$ +42° [lit. (6) mp 214–215°, $[\alpha]_D$ +41°].

Anal.—Calc. for $C_{32}H_{52}O_2$: C, 82.02; H, 11.11. Found; C, 81.96; H, 11.25.

Formation of 29-norlupan-20-one-3β-yl acetate (VIII), by reaction of lupenyl acetate with ozone, showed the position of a C-19 methine proton in the NMR spectrum at δ 3.70 ppm as a quartet (J = 4.6 Hz).

To a solution of 0.1 g (0.02 mole) of lupenyl acetate in 10 ml of absolute ethanol was added a solution containing 0.02 g (0.036 mmole) of potassium hydroxide in 10 ml of 70% ethanol. The mixture was heated at reflux for 4 hr. The solvent was removed on the flash evaporator, and 20 ml of water was added. Lupeol was extracted with methylene chloride.

The methylene chloride was evaporated on the flash evaporator, and the remaining white solid was recrystallized from acetone. The white crystals were dissolved in petroleum ether and chromatographed on 50 g of basic alumina. Elution with benzene–absolute ethanol (5:1) gave lupeol. Lupeol was recrystallized from acetone to give fine white needles, mp 210–211°, $[\alpha]_D^{25}$ +30° [lit. (16) mp 210–212°, $[\alpha]_D^{20}$ +26.4°]. Lupeol (0.08 g, 0.019 mmole) was obtained in 94% yield.

The filtrate from the acetylation that led to the formation of Fraction IA was evaporated by removal of the solvent on the flash evaporator. The yellow flaky residue was recrystallized from 95% ethanol using decolorizing activated charcoal. Further recrystallizations from acetone yielded butyrospermyl acetate (0.41 g), mp 139–140°, $[\alpha]_D^{25}$ +12° [lit. (6) mp 142.5–144°, $[\alpha]_D$ +10°].

The IR spectrum was identical with that of the product of acetylation of an authentic sample of butyrospermol, and there was no depression of melting point upon admixture of that acetylation product. The mass spectrum indicated m/e 468.

To a solution of 20 mg (0.04 mmole) of butyrospermyl acetate in 10 ml of 95% ethanol was added 10 mg (0.25 mmole) of sodium hydroxide in 10 ml of 70% ethanol. The resulting solution was heated to reflux for 2 hr. The solvent was evaporated, water was added, and the butyrospermol was extracted with ether several times. The ether fraction was evaporated to dryness, and the resulting solid was recrystallized several times from methanol, mp 112–113°, $[\alpha]_D^{25}$ −12° [lit. (6) mp 111–112°, $[\alpha]_D^{25}$ −13°].

It was identical with authentic butyrospermol and showed no depression of melting point upon admixture of an authentic sample. The IR spectra were identical.

Anal.—Calc. for $C_{30}H_{50}O$: C, 84.50; H, 11.74. Found: C, 84.30; H, 11.58.

Fraction IB—Removal of petroleum ether and benzene from Fraction IB yielded a brown-colored tar. Recrystallization of the substance from acetone yielded a tan-colored solid, which was collected on a büchner funnel, washed with acetone–water, and then allowed to dry. This procedure gave 0.2 g of Fraction IB.

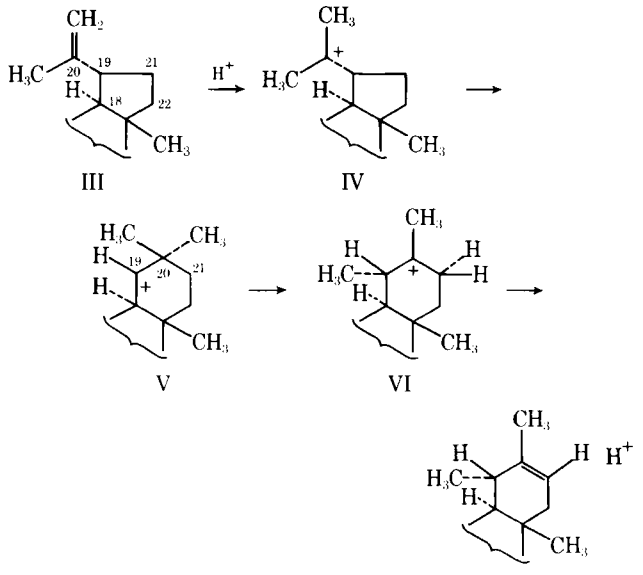
Fraction IB (2.0 g) was chromatographed on 100 g of basic alumina. Elution with petroleum ether–benzene (1:1) gave a fraction, which was recrystallized from acetone and then 95% ethanol to give a solid, mp 180–182°. Treatment with 5 ml of pyridine and 10 ml of acetic anhydride, heating at reflux for 10 min, gave a product which crystallized upon standing at room temperature for 1 hr. The product was recrystallized from 95% ethanol, mp 193–195°, in a yield of 1.5 g (corresponding to 0.38% of the dried fruit).

The IR spectra of the products from Fractions IA and IB showed the presence of a vinylidene group. Both spectra were similar with lupeol (Ia) and lupenyl acetate (Ib), respectively.

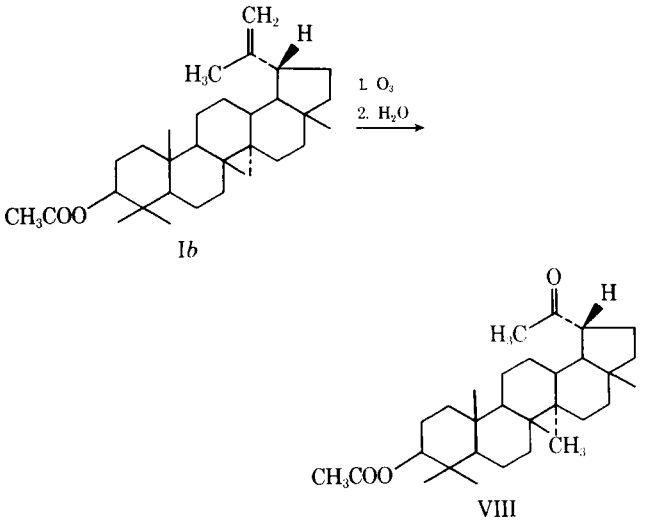
*Separation of 19α-H-Lupenyl Acetate (II*b)—The acetylated product (1.2 g) was chromatographed on 200 g of basic alumina. It was eluted with petroleum ether–benzene (4:1), and 15-ml fractions were collected. The last two fractions (14 and 15) (87 mg, 0.021% of the dried fruit) were combined and recrystallized from 95% ethanol to yield a solid, mp 210–211.5°, $[\alpha]_D^{25}$ +15° (dioxane). The mixed melting point with an authentic sample of lupenyl acetate was depressed to 193°.

There were absorption bands in the IR that indicated the presence of the vinylidene group (3100, 1650, and 880 cm^{-1}); NMR ($CDCl_3$): δ 0.84 (s, 9, 4α, 4β, 10β-CH_3), 1.027 (s, 3, 8β-CH_3), 0.929 (s, 3, 14α-CH_3), 0.780 (s, 3, 17β-CH_3), 2.03 (s, 3, acetoxyl-CH_3), 1.681 (s, 3, 30-CH_3), 4.60 (d, 2, J = 0.1 Hz, vinyl protons on C-29), and AB pattern at δ 4.46 (q, 1, J = 6 Hz, 3α-H); mass spectrum m/e (relative intensity) of major peaks (70 ev): 468 (100) M^+, 453 (28.6) (M − CH_3), 408 (18.5) (M − CH_3COOH), 393 (19.0) [M − (CH_3COOH + CH_3)]: 468 (100), 453 (27.6), 408 (14.9), 393 (8.6); (13 ev): 468 (100), 453 (26.0), 408 (10.4), 393 (7.8); and (10 ev): 468 (100) and 453 (29.2). Formation of the methyl ketone (IX) by reaction with ozone indicated the C-19 methine proton in the NMR spectrum as a quartet at δ 4.46.

Anal.—Calc. for $C_{32}H_{52}O_2$: C, 82.05; H, 11.11. Found: C, 81.67; H, 11.37.



Scheme II—Mechanism of formation of Ψ-taraxasterol (VII) from lupeol (III)



Scheme III—Formation of 29-norlupan-20-one-3β-yl acetate (VIII) by reaction of lupenyl acetate with ozone

Fractions 9 through 13, thought to be a mixture, were combined and recrystallized from 95% ethanol, mp 193–197°. Combined fractions 14 and 15 were again eluted on basic alumina with petroleum ether–benzene (4:1) and gave white crystals, eluting identically.

To a solution of 10 mg (0.02 mmole) of 19α-H-lupenyl acetate in 10 ml of 95% ethanol was added 10 mg (0.18 mmole) of potassium hydroxide in 10 ml of 70% ethanol. The resulting mixture was heated to reflux for 3 hr. The solvent was removed on the flash evaporator, 10 ml of water was added, and 19α-H-lupeol was extracted with ether several times. The ether fraction was evaporated to dryness, and the white solid remaining was recrystallized from acetone and then 95% ethanol, mp 204–206°, $[\alpha]_D^{25}$ +10°.

Anal.—Calc. for $C_{30}H_{50}O$: C, 84.50; H, 11.11. Found: C, 84.42; H, 11.39.

The mass spectrum indicated *m/e* 426; NMR ($CDCl_3$): δ 0.76 (s, 3, 4, CH_3), 0.828 (s, 3, 4β-CH_3), 0.960 (s, 3, 10β-CH_3), and 3.15 (q, 1, J = 6.0 Hz, 3α-H).

Fraction IC—Removal of the solvent from Fraction IC yielded a tar-like substance, which was washed with acetone and then recrystallized from 95% ethanol using activated charcoal. The product (0.08 g) was chromatographed on 100 g of basic alumina with benzene–absolute ethanol (3:1) to give an orange-colored viscous oil (15 mg). Treatment with pyridine (3 ml) and acetic anhydride (10 ml) overnight gave a monoacetate which, after recrystallization with acetone and then 95% ethanol, gave needles, mp 253–255°, $[\alpha]_D^{25}$ +16° [lit. (6) mp 248.5–250°, $[\alpha]_D^{25}$ +13.5°].

There was no depression of melting point upon admixture of an authentic sample of lupane-3β,20-diol 3-monoacetate. The IR spectrum was identical with that of an authentic sample. There was a sharp OH stretch at 3520 cm^{-1}, C=O stretch at 1720 cm^{-1}, and C—O stretch at 1250 cm^{-1}. The mass spectrum gave *m/e* 486.

To a solution of 0.3 g (0.06 mmole) of lupane-3β,20-diol 3-monoacetate in 10 ml of 95% ethanol was added 0.1 g (0.18 mmole) of potassium hydroxide in 20 ml of 70% ethanol. The solution was heated in reflux for 30 min, and the solvent was distilled off. Water (10 ml) was added to the white-colored residue and the product was extracted with ether. After distillation of the ether, the product was chromatographed on basic alumina (100 g) with benzene–absolute ethanol (1:1) to yield lupane-3,20-diol, mp 238–240°, $[\alpha]_D^{25}$ +3.9° [lit. (6) mp 238–241°, $[\alpha]_D$ +4°].

Reaction of Ib with Ozone—Compound Ib (1.0 g, 0.21 mmole) in 20 ml of chloroform was treated with a slow stream of ozone at 10° for 1 hr. Completion of the formation of the ozonide was ensured by inserting the outlet tube into a 5% potassium iodide solution. The solvent was removed *in vacuo* at room temperature, leaving the transparent glass-like ozonide to which 100 ml of water was added. The mixture was heated for 1 hr and then steam distilled. To the distillate was added an alcoholic solution of 2,4-dinitrophenylhydrazine. A yellow solid formed, which was collected on a büchner funnel and allowed to dry. The yellow solid was recrystallized from 95% ethanol to give needles, mp 163° [lit. (7) mp 166°]. There was no depression of the melting point upon admixture of an authentic sample of formaldehyde 2,4-dinitrophenylhydrazone. The hydrazone derivative corresponded to a 30% yield of formaldehyde.

The residue from the steam distillation was evaporated to yield a white solid, which was extracted with ether and washed several times with 10% $NaHCO_3$. The ether was removed and the product was recrystallized three times from acetone and then twice from 95% ethanol to yield white needles, mp 255–257°, $[\alpha]_D^{25}$ +9.8° [lit. (9) mp 258–260°, $[\alpha]_D^{20}$ +8.3°]. 29-Norlupan-20-one-3β-yl acetate (0.3 g, 0.063 mmole) was obtained in a 30% yield.

The IR spectrum indicated loss of the peaks corresponding to the vinylidene group in lupenyl acetate at 3100, 1650, and 880 cm^{-1}. The carbonyl ketone group showed a peak at 1695 cm^{-1}. NMR ($CDCl_3$): δ 0.84 (s, 9, 4α, 4β, 10β-CH_3), 1.02 (s, 3, 8β-CH_3), 0.960 (s, 3, 14α-CH_3), 0.764 (s, 3, 17β-CH_3), 2.143 (s, 30-CH_3), 2.03 (s, 3, acetoxyl-CH_3), *AB* pattern at δ 4.46 (q, 1, J = 6.0 Hz, 3α-H), and 3.70 (q, 1, J = 4.6, Hz, C-29 H); mass spectrum *m/e* (relative intensity) of major peaks (70) ev): 470 (24.1) (M^+), 455 (1.9) (M − CH_3), 440 (2.8) (M − $2CH_3$), 425 (1.9) (M − $3CH_3$), 410 (100) (M − CH_3COOH), 395 (29.6) [M − (CH_3COOH + CH_3)], 377 (3.7), 367 (44.6), 354 (6.5), 341 (13.9), and 328 (13.9).

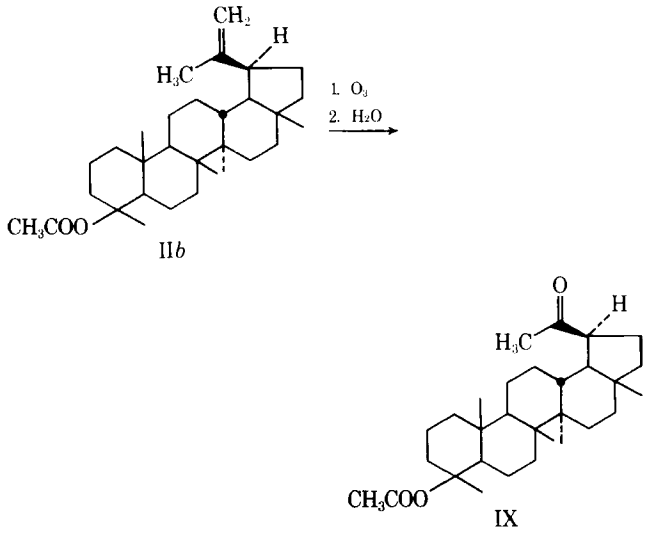
Anal.—Calc. for $C_{31}H_{50}O_3$: C, 79.15; H, 10.64. Found: C, 78.97; H, 10.50.

Reaction of 19α-H-Lupenyl Acetate with Ozone—Following the procedure for the ozonolysis of lupenyl acetate, 29-nor-19α-H-lupan-20-one-3β-yl acetate was formed from the reaction of ozone with 19α-H-lupenyl acetate. The product was recrystallized three times with acetone and then two times with methanol, mp 249–251°, $[\alpha]_D^{25}$ +43°.

IR spectra showed the loss of peaks, indicating the presence of the vinylidene group at 3100, 1650, and 880 cm^{-1}. The ketone carbonyl group appeared at 1690 cm^{-1}. NMR ($CDCl_3$): δ 0.84 (s, 9, 4α, 4β, 10β-CH_3), 1.02 (s, 3, 8β-CH_3), 0.949 (s, 3, 14α-CH_3), 0.749 (s, 3, 17β-CH_3), 2.143 (s, 3, 30-CH_3), 2.03 (s, 3, acetoxyl-CH_3), *AB* pattern at δ 4.46 (q, 1, J = 6 Hz, 3α-H), and 4.10 (q, 1, J = 4.8 Hz, C-29 H); mass spectrum *m/e* (relative intensity) of major peaks (70 ev): 470 (24.0) (M^+), 455 (2.6) (M − CH_3), 440 (1717) (M − $2CH_3$), 425 (5.3) (M − $3CH_3$), 410 (100) (M − CH_3COOH), 395 (30.3) (M − CH_3COOH), 377 (4.4), 367 (49.3), 354 (6.6), 341 (13.9), and 328 (13.9).

Anal.—Calc. for $C_{31}H_{50}O_3$: C, 79.15; H, 10.64. Found: C, 78.90; H, 10.57.

Equilibration of 29-Norlupan-20-one-3β-yl Acetate (VIII) with Sodium Ethoxide—A solution of 65 mg (0.14 mmole) of VIII in 10 ml of absolute ethanol was added to a solution of 26 mg (0.4 mmole) of sodium ethoxide in 10 ml of absolute ethanol. The solution was stirred at room temperature for 24 hr. The solvent was re-



Scheme IV—Formation of 29-nor-19α-H-lupan-20-3β-yl acetate (IX) by reaction of 19α-H-lupenyl acetate with ozone

moved on the flash evaporator. To the resulting brown solid, 40 ml of water was added. The mixture was stirred at room temperature for 2 hr and then extracted with ether.

The ether was evaporated on the flash evaporator, and to the resulting product was added 10 ml of a solution of acetic anhydride–pyridine (5:1). The mixture was heated for 10 min and then allowed to stand at room temperature for several days, during which time crystals formed. The crystals were collected and recrystallized three times from acetone and then twice from methanol to give 50 mg (76%) of a solid, mp 250–251°, $[\alpha]_D^{25}$ +43°.

Anal.—Calc. for $C_{31}H_{48}O_3$: C, 79.15; H, 10.64. Found: C, 78.90; H, 10.57.

The product was identical with the product of reaction of 19α-H-lupenyl acetate with ozone and showed no depression of melting point upon admixture of that product.

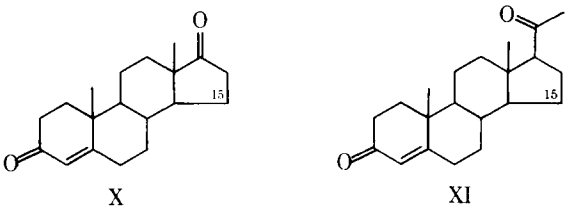
Conversion of Lupeol (Ia) to Ψ-Taraxasteryl Acetate (VII)—Following the procedure of Halsall *et al.* (13), lupeol (3.0 g, 0.7 mmole) was converted to Ψ-taraxasterol, which was reacted with acetic anhydride (10 ml) and pyridine (5 ml). The product was recrystallized from 95% ethanol several times and eluted over 100 g of basic alumina with petroleum ether–benzene (1:1) to give Ψ-taraxasteryl acetate (0.1 g, 20% yield), which was isolated as long white needles, mp 243–244°, $[\alpha]_D^{25}$ +62° [lit. (9) mp 238–240°, $[\alpha]_D^{25}$ +56°].

Conversion of 19α-H-Lupeol (IIa) to Ψ-Taraxasteryl Acetate (VII)—Following the procedure used in the conversion of lupeol to Ψ-taraxasteryl acetate, 19α-H-lupeol (40 g, 0.09 mmole) was converted to the identical product, mp 241–242°. There was no depression of melting point upon admixture of Ψ-taraxasteryl acetate; IR and mass spectra were identical.

DISCUSSION

Structural Characterization of Epimeric 19α-H-Lupenyl Acetate (IIb)—The lupane carbon skeleton contains 10 areas of potential dissymmetry including C-3; the carbon atoms comprising ring junctures C-5 and C-10, C-8 and C-9, C-13 and C-14, and C-17 and C-18; and the carbon atom at which the isopropenyl group is located, C-19. Acidic isomerization of 19α-H-lupeol (IIa), under the conditions specified by Halsall *et al.* (13), produced the identical pentacyclic triterpene as produced by lupeol (Ia), Ψ-taraxasterol (see partial structure VII, Scheme II), which was isolated as the 3-acetate.

From the mechanism proposed by Halsall *et al.* (13) (Scheme II), the only asymmetric site disturbed by these conditions is C-19. The first step predicts protonation of C-19 of partial structure III, leading to the formation of carbonium ion IV with the positive center located at C-20. Ring enlargement follows to form carbonium ion V, which places the positive charge at C-19. Migration of the α-axial methyl group on C-20 leads to carbonium ion VI which, after loss of a proton, gives Ψ-taraxasterol (VII). Not precluding the possibility of another isomer or mechanism leading to formation of VII, identical structures for the two epimers I and II in rings A, B, C, and D were indicated.



Prominent peaks for lupenyl acetate (Ib) and 19α-H-lupenyl acetate (IIb) in their NMR spectra were similar. Major differences in the spectra were uninterpretable occurring in the methine area at δ 1.59–1.65 ppm. To shift C-19 protons of Ib and IIb irrespectively away from interference, both Ib and IIb were treated with ozone to yield the corresponding ketones, 29-norlupan-20-one-3β-yl acetate (VIII) and 29-nor-19α-H-lupan-20-one-3β-yl acetate (IX) (Schemes III and IV).

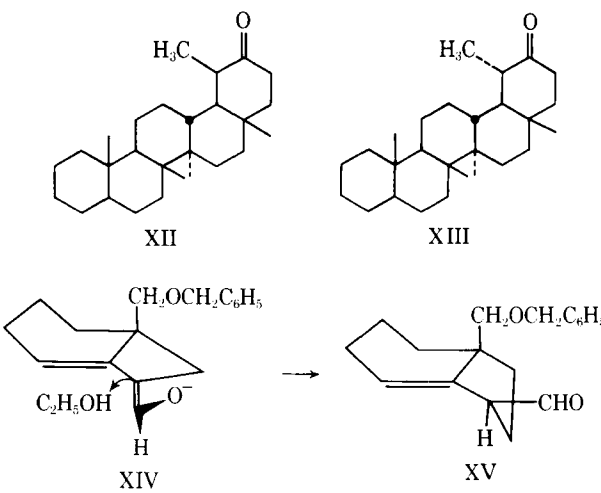
Ketone formation with ozone was used to avoid possible disturbance of the asymmetry of C-19, such as in oxidation in acidic media with chromic anhydride (8). However, acidic by-products are formed by ozone reaction (13). Previously, the ketone purification was performed by removal of acids by extraction with 10% potassium hydroxide (8). To prevent epimerization at C-19, this was avoided by use of sodium bicarbonate.

The NMR spectrum of VIII indicated a methine proton at δ 3.70 ppm, J = 4.6 Hz, attributed to the C-19 methine proton. It is adjacent to the carbonyl and would be expected to shift downfield. The methine proton appeared as a quartet (δ 3.82, 3.74, 3.67, and 3.59), in which first-order rules could be applied for its interpretation. The proton is in the environment of three nonequivalent protons, so four peaks would be expected.

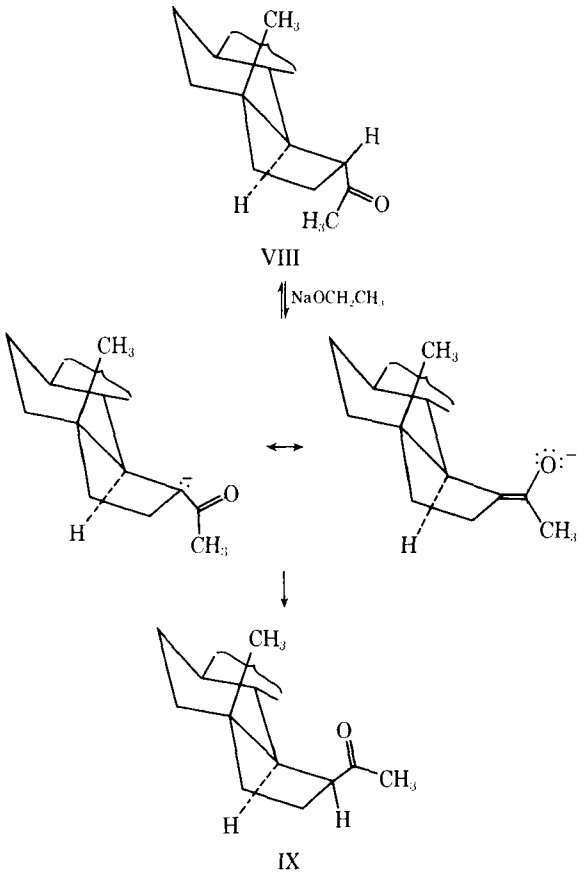
If Ib and IIb were epimeric at C-19, ozonolysis of the latter should yield Compound IX. The NMR spectrum of its product (IX) indicated the C-19 methine proton at δ 4.10, J = 4.8 Hz, as a quartet (δ 4.22, 4.14, 4.06, and 3.98), in which first-order rules were applied for its interpretation. No other proton in Ib could show a quartet in which the coupling constant is about 4.8 Hz unless that proton were at C-19.

The chemical shift difference between the two C-19 quartets of VIII and IX is δ 0.40. The difference in chemical shift compared to measurements obtained from 15-substituted hydroxy and acetoxy derivatives of Δ^4-androstene-3,17-dione (X) and Δ^4-pregnene-3,20-dione (XI) (13). The C-15 position of the five-membered rings in X and XI can correspond to substituents of C-19 in I or II. In both sets of molecules, each site in the five-membered ring is the third carbon atom from the β-methyl at the ring junction and the first carbon atom from the α-hydrogen at the ring juncture; they are, therefore, models for comparison.

The chemical shifts of the protons attached to the same carbon



Scheme V



Scheme VI—Formation of 29-nor-19H-lupan-20-3-yl acetate (VIII) from 29-norlupan-20-one-3-yl acetate (IX) with sodium ethoxide, followed by dilution with water and reesterification of the C-3 hydroxy group

atom within the skeletons of X and XI are characteristic of the position and configuration in which these protons are situated (13). There is a high field shift of an axial proton relative to its equatorial counterpart when these compounds are substituted alcohols. The chemical shift difference for the C-15 methine protons is δ 0.20 + 0.03; for the corresponding 15-substituted acetates, it is δ 0.20 (13).

Epimerization of ketone VIII to IX occurred with sodium ethoxide. The strong base affected only the asymmetry of one carbon atom in VIII, C-19. The epimerization of VIII to IX is similar to the epimerization of 30-nortaraxastan-20-one (XII) to 30-nor-19α-H-taraxastan-20-one (XIII), when absorbed from benzene onto neutral alumina [Activity I, pH 7–8 (14)]. In addition, Marshall and Greene (14) indicated the stereochemical outcome of the reaction of the enolate of 7a-benzyloxymethyl-2,3,4,5,6,7,7a-hexahydroindene-3-carboxaldehyde (XIV) with ethanol. Protonation of the enolate by ethanol occurred from the less hindered side to give the β,γ-unsaturated aldehyde (XV), in which the carboxaldehyde group is in the β-axial orientation (Scheme V).

Upon equilibrating VIII with sodium ethoxide, followed by dilution with water, extraction with ether, and reesterification with acetic anhydride–pyridine, only one pure ketone (IX) was isolated (Scheme VI). Where a ring is rigid, such as in steroidal ketones, addition reactions to the carbonyl function proceed from the equatorial side (15). As illustrated in Scheme VI, after removal of the C-19 proton, the asymmetry at that site is disturbed. The configuration at C-19 of the final product IX is determined in the addition step, in which the proton adds from the sterically more accessible side equatorially. Therefore, the acetyl group of the final product IX is exclusively in the β-axial orientation.

Since the product of equilibrium of VIII in base gave IX and this was identical with the product of ozonation of II*b* to IX (Scheme IV), it then follows that the difference between I*b* and II*b* is solely at C-19.

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