# Structure of Lupeol and Its 19α-H-Isomer

J. E. GEARIEN ˣ, L. BAUER, M. KLEIN, and B. LEVINE

**Abstract** □ The silver-ion-assisted dehydrochlorination rearrangement of 19α-chloro-18α-oleanane-3β-ol (lupeol hydrochloride) was reinvestigated. The alkene so obtained was ozonized to yield an epimeric mixture (at C-19) of two methyl ketones. The mechanism of the rearrangement of the chloro derivative to lupeol and its 19α-H-isomer is reinterpreted in the light of these new findings.

**Keyphrases** □ Lupeol and 19α-H-isomer—structure determination □ 19α-Chloro-18α-oleanane-3β-ol (lupeol hydrochloride)—structure determination

The isolation of 19α-H-lupeol (Ia) from the fruit of the Osage orange *(Maclura pomifera)* (1) prompted a reexamination of the method exployed by Halsall *et al.* (2) in assigning the configuration of lupeol (IIa) at C-19. These investigators based their conclusion on the dehydrochlorination of 19α-chloro-18α-oleanene-3β-ol (IIIa, lupeol hydrochloride) to furnish lupeol. This reaction involved the elimination of hydrogen chloride with the simultaneous ring contraction from a six- to a five-membered ring. The conclusions reached by Halsall *et al.* were predicated on the assumption that a set of stereospecific reactions was involved in the overall transformation.

## DISCUSSION

Halsall *et al.* set out to determine the configuration at C-19 of lupeol in the following manner. First, lupeol was treated with ethanolic hydrogen chloride solution at room temperature to produce IIIa. They determined that the chloro group in this new product is in the equatorial position and that H-19 is then axial (2). Since the product of their dehydrohalogenation was not the alkene germanicol but rather lupeol, they reasoned that the normal type $E_2$ *trans*-elimination of hydrogen chloride was not possible.

Instead, Halsall *et al.* (2) suggested a kind of stereospecific displacement of the chloro group by a neighboring methylene group, which would be equivalent to a stereospecific ring contraction, to lead to an isopropyl carbonium ion. This is reproduced, as they conceived it in Scheme I. This overall conversion is analogous to a number of similar ring contractions of ring A in various triterpenes as, for example, the reaction of the methanesulfonate of cholestanol to give 3β-isopropyl derivatives (3). Since Halsall *et al.* (2) isolated only one isomer from IIIa and confirmed it to be lupeol, they used the mechanism in their Scheme I as evidence for assigning the configuration of the proton at C-19 in IIa[1].

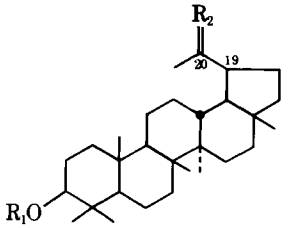
In repeating the work of Halsall *et al.*, IIa was converted first to IIIa. Dehydrohalogenation and subsequent acetylation, using their procedure, gave a mixture which consisted of Ib and IIb. The prime data used by Halsall *et al.* to identify IIa were the melting point and optical rotation values. In this study, PMR spectroscopy was used to examine the product further.

The C-19 methine protons in this mixture were obscured by the envelope of PMR signals due to the other methylene and methine protons. To analyze the mixture more meaningfully, the structure would have to be modified to shift H-19 downfield [from $(CH_3)_4Si$]. Such an anisotropic shift was realized when Ib and IIb were converted to the corresponding ketones, V and IV, respectively. To accomplish this, the reaction mixture from the rearrangement was treated with ozone. A mixture of ketones, 3β-acetoxy-30-norlupan-20-one (IV) and its α-H, at C-19, isomer (V) was obtained.

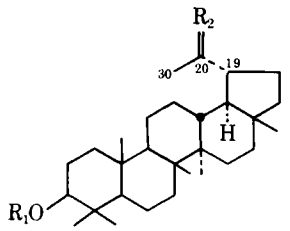
Extreme care was exercised to avoid strong base during the work-up to prevent epimerization. It was then possible to examine the PMR spectrum for the presence of these isomers. The PMR spectrum of the product of the ozonolysis indicated that a mixture contained the epimeric ketones at C-19 in the ratio of approximately 1:1. Two quartets were observed in this PMR spectrum: δ 3.71 ($J$ = 4.20 Hz) and 4.10 ($J$ = 4.80 Hz). These quartets and the rest of the spectrum paralleled the chemical shifts and coupling constants reported previously for IV and V, respectively (1).

It appears that the rearrangement of IIIa yields lupeol and its 19α-epimer. A synchronous process could be accommodated if ring E in IIIa is more in a skewed conformation than was assumed by Halsall *et al.* (2). The bulky substituents surrounding the chloro group might force considerable distortion of ring E and, in effect, force the ring to be more planar. Djerassi *et al.* (4) noted that some compounds that contain a *gem*-dimethyl grouping, as at C-20 in III, in the vicinity of the chromophore showed inverted rotatory dispersion curves, attributed to conformational distortion of the system. Thus, the attacking methylene carbon could displace the chloro group either from above or below the plane of that ring.

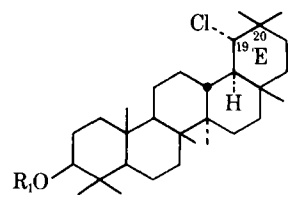
An alternative explanation is offered. Abstraction of chloride ion by silver ion usually creates an incipient carbonium ion, which




Ia: $R_1$ = H, $R_2$ = $CH_2$
Ib: $R_1$ = $COCH_3$, $R_2$ = $CH_2$
V: $R_1$ = $COCH_3$, $R_2$ = O

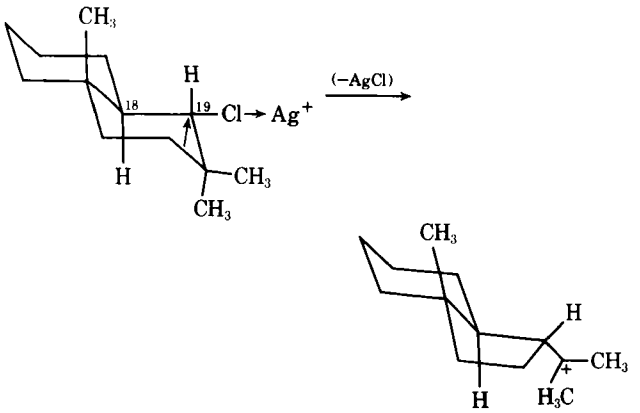



IIa: $R_1$ = H, $R_2$ = $CH_2$
IIb: $R_1$ = $COCH_3$, $R_2$ = $CH_2$
IV: $R_1$ = $COCH_3$, $R_2$ = O

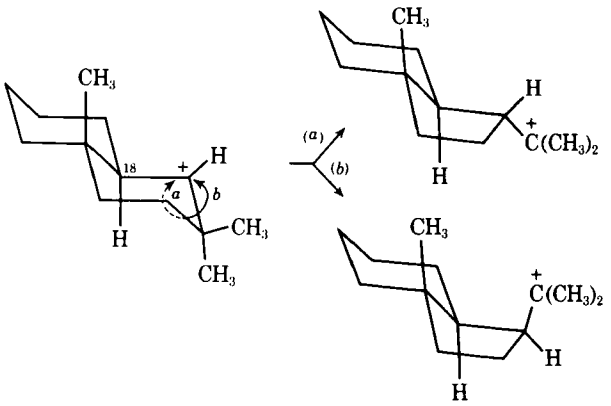



IIIa: $R_1$ = H
IIIb: $R_1$ = $COCH_3$

[1] While this work was in progress, the configuration at C-19 was settled by the total synthesis of lupeol [G. Stork, S. Uyeo, T. Wakamatsu, P. Grieco, and J. Labovits, *J. Amer. Chem. Soc.*, **93**, 4945(1971)]. Its stereochemistry was important in assignment of related triterpenoids. See A. S. Samson and R. Stevenson, *Org. Prep. Proced.*, **5**, 59(1973); and E. Klinotova, N. Hovorkova, J. Klinot, and A. Vystrcil, *Collect. Czech. Chem. Commun.*, **38**, 1179(1973).



*Scheme I—$E_2$ trans-elimination of 19α-chloro-18α-oleanane-3β-ol when ring E is represented in the chair conformation*



*Scheme II—Ring contraction via carbonium ion at C-19*

then would be expected to produce two plausible rearrangement products (Scheme II). Loss of a proton from the carbonium ion would then furnish IIa (from path *a*) and IIb (from path *b*).

## EXPERIMENTAL[2]

**Materials**—The silica gel powder[3] used was 60–200 mesh in size.

Chromatographic solvents were distilled prior to use. The petroleum ether used for chromatography, as well as in the extraction procedure, had a boiling range of 40–60°.

**Preparation of Chromatographic Columns**—The column[4] used was 500 × 50 mm. The chromatographic columns were prepared by the slurry method. Benzene and silica gel powder were mixed into a slurry, and the slurry was then poured into the column and allowed to settle. Petroleum ether was then passed through the column until all of the benzene was removed.

**Extraction of Lupeol (Ia)**—Dry lupini beans[5] (the seed of *Lupinus* spp.) (500 g) were soaked overnight in water. On the following day the testae from the seeds were removed and placed in an oven for 12 hr at 100°. The dried testae were then ground in an electric blender until a fine powder was obtained. This procedure was repeated 25 times, until a total of 12.5 kg of lupini beans was used. From this amount, 2000 g of dried testa powder was collected.

The dried testa powder (1000 g) was extracted with petroleum ether for 72 hr in a soxhlet extraction apparatus. The defatted powdered material was then replaced with the remaining 1000 g, and the extraction process was repeated.

Evaporation of the extract yielded a light-yellow solid (14.0 g).

The solid (1.0 g) was dissolved in a minimum amount of chloroform. This solution was mixed with dry silica gel powder (2.0 g) and placed in an oven for 12 hr at 100°. The resulting mixture was then layered at the top of a packed chromatographic column.

The column was eluted with: *(a)* petroleum ether (1000 ml), *(b)* petroleum ether–benzene (4:1, 1000 ml), *(c)* petroleum ether–benzene (2:1, 500 ml), and *(d)* petroleum ether–benzene (1:1, 500 ml). The column was then eluted exhaustively with benzene.

Evaporation of the benzene fraction yielded a white powder, which was recrystallized twice from acetone and once from 95% ethanol to yield lupeol as white needles, mp 210–212° [lit. (5) mp 211–212°]. Lupeol (9.0 g) was obtained in 0.45% yield based on the dry lupini bean.

The IR spectrum was identical with that of an authentic sample. The melting point was undepressed upon admixture of an authentic sample.

**19α-Chloro-18β-oleanane-3β-ol (IIIa)**—Dry ethanol (800 ml) was saturated with hydrogen chloride gas at 0° and gradually added, with periodic cooling, to a solution of lupeol (9.0 g, 0.021 mole) in dry ethanol (500 ml). The mixture was allowed to stand for 5 days at 20° and then diluted with water (700 ml), and extraction with chloroform yielded the product. Two recrystallizations from 95% ethanol yielded needles (3.80 g), mp 206–208°.

Further purification was effected by chromatographing the product (0.76 g) on a silica gel column, prepared in benzene–petroleum ether (1:4). Elution with a mixture of benzene–ether (1:1, 500 ml) yielded a fraction, which was recrystallized twice from 95% ethanol to give needles (0.58 g), mp 210–212° [lit. (2) mp 211–212°]. This chromatographic procedure was repeated five times, and 19α-chloro-18α-oleanane-3β-ol (2.90 g, 0.0063 mole) was obtained in a 32.2% yield. The IR spectrum indicated the loss of the characteristic vinylidene bands at 3100, 1650, and 880 $cm^{-1}$.

*Anal.*—Calc. for $C_{30}H_{51}ClO$: C, 77.84; H, 11.03; Cl, 7.68. Found: C, 77.76; H, 10.99; Cl, 7.75.

**Dehydrochlorination and Acetylation of IIIa**—19α-Chloro-18α-oleanane-3β-ol (2.90 g, 0.0063 mole) was dissolved in hot 95% ethanol (300 ml) and heated at reflux for 20 hr with silver acetate (4.0 g). During this time the suspended solid darkened and a silver mirror formed on the surface of the flask. The mixture was then diluted with water (500 ml) and extracted with ether. Ether was evaporated, and the remaining solid was refluxed for 2 hr with acetic anhydride (35 ml).

This solution was then poured into water (250 ml), and the aqueous solution was extracted with ether. After removal of ether, the residue was recrystallized twice from 95% ethanol to yield white needles, mp 206–208° [lit. (2) mp 215–216° for lupenyl acetate, lit. (1) mp 210–211.5° for 19α-H-lupenyl acetate]. The product (2.10 g, 0.0043 mole) was obtained in a 72.4% yield.

The IR spectrum indicated the presence of a vinylidene group by the presence of peaks at 3100, 1650, and 880 $cm^{-1}$. The broad O—H stretching band in the starting alcohol had disappeared, with the appearance of a C—O stretching band at 1240 $cm^{-1}$, and a C=O stretching band appeared as a sharp peak at 1750 $cm^{-1}$.

*Anal.*—Calc. for $C_{32}H_{52}O_2$: C, 82.02; H, 11.11. Found: C, 81.97; H, 11.19.

**Ozonolysis of Dehydrochlorinated and Acetylated Products (IV and V) from IIIa**—A solution of lupenyl acetate and/or its epimer (2.10 g, 0.0043 mole) in chloroform (40 ml) was treated at 10° with a slow stream of ozone for 1 hr. Completion of formation of the ozonide was ensured by inserting the outlet tube into a 5% potassium iodide solution. Immediate oxidation of the iodide indicated that the reaction was complete.

The solvent was evaporated, leaving the ozonide as an oil. Water (200 ml) was added, and the mixture was heated for 1 hr and then steam distilled. To the distillate was added an alcoholic solution of 2,4-dinitrophenylhydrazone.

After the steam distillation, the aqueous phase was extracted with ether. Removal of ether furnished a product, which was re-

[2] Melting points were determined on a Thomas-Hoover capillary melting-point apparatus and are uncorrected. Elemental analyses for carbon, hydrogen, and chlorine were performed by Micro-Tech Laboratories, Inc., Skokie, Ill. The NMR spectrum was determined using a Bruker, model HFX05, 90-MHz spectrometer and was recorded downfield from tetramethylsilane as an internal reference. The solvent used was deuterated chloroform. IR spectra were obtained with a Perkin-Elmer model 700 recording spectrophotometer. Nujol mulls were used for all samples run. Ozone was generated by passing oxygen through a Welsbach Corp. model T23 ozonizer.

[3] J. T. Baker Chemical Co., Phillipsburg, N.J.

[4] Kontes, Vineland, N.J.

[5] Purchased from Conti-Di-Savoia European Specialties, Chicago, Ill.

crystallized twice from aetone and then twice from 95% ethanol to yield white needles, mp 244–246° [lit. (5) mp 260–262° for 29-norlupan-20-one-3$\beta$-yl acetate [lit. (5) mp 249–251° for 29-nor-19$\alpha$-H-lupan-20-one-3$\beta$-yl acetate]. The product (0.57 g, 0.0012 mole) was obtained in a 27.0% yield.

The IR spectrum indicated loss of the peaks corresponding to the vinylidene group at 3100, 1650, and 880 $cm^{-1}$. Carbonyl absorption at 1700 $cm^{-1}$ was observed.

*Anal.*—Calc. for $C_{31}H_{50}O_3$: C, 79.15; H, 10.64. Found: C, 79.01; H, 10.50.

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# Synthesis of Substituted Benzylidinohydrazines and Their Monoamine Oxidase Inhibitory and Anticonvulsant Properties


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**Abstract** □ Some $N^1$-(4-acetamidobenzoyl)-$N^2$-(substituted phenyl carboxylate)benzylidinohydrazines were synthesized, characterized, and evaluated for their ability to inhibit monoamine oxidase *in vitro*. All substituted benzylidinohydrazines inhibited monoamine oxidase activity of rat brain homogenates. These compounds possessed anticonvulsant activity, which was reflected by the protection afforded against pentylenetetrazol-induced convulsions; they also potentiated pentobarbital-induced hypnosis in mice. Monoamine oxidase inhibitory effectiveness of these substituted benzylidinohydrazines was unrelated to their anticonvulsant activity and their ability to potentiate pentobarbital-induced hypnosis.



**Keyphrases** □ Benzylidinohydrazines—synthesis, characterization, and *in vitro* inhibition of monoamine oxidase □ Structure–activity relationships—benzylidinohydrazines, anticonvulsant activity and inhibition of monoamine oxidase activity of rat brain homogenates □ Monoamine oxidase inhibitors—synthesis of benzylidinohydrazines □ Anticonvulsant activity—benzylidinohydrazines


Many hydrazine derivatives are monoamine oxidase inhibitors (1). Such enzyme inhibitors have been shown to possess anticonvulsant properties (2). Furthermore, psychotropic (3) and anticonvulsant (4) properties exhibited by benzylidine derivatives led to the synthesis of $N^1$-(4-acetamidobenzoyl)-$N^2$-(substituted phenyl carboxylate)benzylidinohydrazines. The ability of these benzylidinohydrazines to inhibit monoamine oxidase activity of rat brain homogenates was investigated in an attempt to correlate enzyme inhibitory effectiveness with anticonvulsant activity and ability to potentiate pentobarbital-induced hypnosis.

## EXPERIMENTAL

**Chemistry**—*Ethyl 4-Aminobenzoate*—Ethyl 4-aminobenzoate was prepared by the esterification of 4-aminobenzoic acid by the method reported earlier, mp 91° (5).

*Ethyl 4-Acetamidobenzoate*—In a conical flask containing water, 18.3 ml of concentrated hydrochloric acid and 35 g of ethyl 4-aminobenzoate (0.22 mole) were introduced with stirring. To this solution, 256 ml of distilled acetic anhydride was added. The reaction mixture was added to a solution of 33 g of sodium acetate in 100 ml of water and stirred vigorously. On cooling, the solid that separated was filtered, washed, dried, and recrystallized from ethanol, mp 117°.

*(4-Acetamidobenzoyl)hydrazine*—A mixture of ethyl 4-acetamidobenzoate (0.4 mole) and 0.4 mole of hydrazine hydrate (99–100%) in absolute ethanol was refluxed on a steam bath for 15 hr. Excess ethanol was distilled, and the hydrazine that separated on cooling was filtered and recrystallized from ethanol, mp 277°.

$N^1$-*(4-Acetamidobenzoyl)*-$N^2$-*substituted Benzylidinohydrazines*—A mixture of 4-acetamidobenzoylhydrazine (0.1 mole) and a suitable salicylaldehyde (0.1 mole) in ethanol with a few drops of acetic acid was refluxed on a steam bath for 4–5 hr. Excess ethanol was removed by distillation. The solid mass that separated was collected by filtration, washed with water, dried, and recrystallized from ethanol. These compounds were characterized by their sharp melting points and elemental analyses (Table I).

$N^1$-*(4-Acetamidobenzoyl)*-$N^2$-*(aryl-substituted Phenyl Carboxylate)benzylidinohydrazines*—$N^1$-(4-Acetamidobenzoyl)-$N^2$-substituted benzylidinohydrazine (0.025 mole) in dry benzene (20 ml) was mixed with the appropriate benzoyl chloride, and the resulting mixture was refluxed on a steam bath for 5–6 hr. Excess benzene was removed by distillation, and the crude product that separated on cooling was filtered, washed first with sodium bicarbonate and then with water, and recrystallized from ethanol. These substituted benzylidinohydrazines, characterized by their sharp melting points and elemental analyses, are recorded in Table II.

**Determination of Monoamine Oxidase Activity**—Male rats weighing 150–200 g were killed by decapitation. Brains were quick-