Structure of Rishitinone, a Valencane Stress Metabolite in Diseased Potato[1)]

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The isolation and structure elucidation of the title valencane sesquiterpene, a stress metabolite produced in diseased potato tubers, are described.

In a recent communication,[2)] we reported the isolation and structure of a valencane sesquiterpene named rishitinone (**1**), a stress metabolite produced in diseased potato tubers. In view of the fact that all stress metabolites so far isolated from the Family Solanaceae differ in planar structure and/or stereochemistry from usual naturally occurring sesquiterpenes, the sesquiterpene (**1**) with the valencane skeleton stands unique on biogenetic grounds as stress compounds.[3)] In the present paper we describe details of the isolation and structure elucidation of the compound.

Isolation. The metabolite was isolated from tuber tissues of potatoes (Rishiri, *Solanum tuberosum*×*S. demissum*) infected with an incompatible race of *Phytophthora infestans* as follows. Neutral chloroform extracts[4)] (83 g), obtained from the diseased potato tubers (300 kg), were separated as described previously[1)] to give "rishitin-rich" fractions (Fraction III,[1)] 5.5 g), which on acetylation followed by chromatography over silica gel afforded a semi-crystalline mixture of acetates. Fractional recrystallization of the mixture gave pure rishitin diacetate[4)] (3.3 g), mp 65—67 °C, and crude oily rishitinone acetate (**1a**) (0.11 g), which was purified by chromatography to yield **1a** (68 mg), oil, $[\alpha]_D$ −2.4°, in pure state. The acetate (**1a**) was then hydrolyzed smoothly to give rishitinone (**1**), mp 72—75 °C, $[\alpha]_D$ +10.1°.

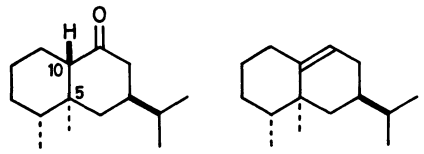
Planar Structure. Rishitinone (**1**) was analyzed for $C_{15}H_{24}O_2$ (*m/e* 236, M^+) and converted by hydrogenation over palladium into dihydrorishitinone (**2**), mp 80—82 °C, $[\alpha]_D$ +7.2°, and by acetylation into the acetate (**1a**). The latter (**1a**) was reduced with sodium borohydride to give hydroxy acetate (**3**), $C_{17}H_{28}O_3$ (*m/e* 280, M^+), mp 96—98 °C, $[\alpha]_D$ −6.9°, as a sole product (90%). The IR and ^{1}H NMR spectra of these compounds indicated the presence of the following structural units: CH_3-(■)-[5)] [**1**, δ 0.76 (3H, s)]: $CH_3\overset{|}{C}H$- [**1**, δ 0.84 (3H, d, J=7 Hz)]: CH_2-$C(CH_3)$- [**1**, IR, 1651 and 900 cm^{-1}; δ 1.76 (3H, s) and 4.77 (2H, br s); **2**, IR, 1388 and 1372 cm^{-1}, δ 0.91 (6H, d, J=6 Hz)]: -C(=O)- [**1**, IR, 1710 cm^{-1}]: -CH(OH)- [**1**, IR, 3500 cm^{-1}; δ 3.65 (1H, br m, W_H=25 Hz); **1a**, IR, 1739 and 1248 cm^{-1}; δ 2.02 (3H, s) and 4.77 (1H, br m, W_H=25 Hz)]. The ^{13}C NMR spectra of **1**, obtained under proton-noise decoupled and single-frequency off-resonance decoupled conditions, provided additional information of the other carbon atoms, four $-CH_2-$ [δ 30.2, 39.3, 42.8, and 46.0 (each t)] and two -CH- [δ 41.3 or 41.5 and 56.1 (each d)], indicating that the ketone (**1**) possesses a bicyclic skeleton.

The ^{1}H NMR spectra of **1** in the presence of the shift reagent Eu(dpm)$_3$ (0.2 and 0.4 mol equiv.) led to downfield shift of all the signals and revealed one-proton signals (W_H=25 Hz) at δ 6.80 and 10.50 in the respective spectra, which were reasonably assigned to the proton on the carbon atom bearing the hydroxyl group. The spectra, coupled with spin-decoupling studies (Table 1), strongly suggested that the ketone (**1**) would probably involve partial formula A. In view of the remaining nine carbon units (formula B) deduced from the afore-mentioned spectra, specially from the ^{13}C NMR spectra [only two (■) at δ 40.7 and 211.1, and one -CH- and one $-CH_2-$ at low field, δ 69.5 and 46.0], formula C was presented as one of the most plausible planar structures for **1**. The following comments on the stereochemistry are based on the formula (C).

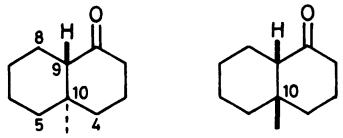
Stereochemistry. The presumption that rishitinone (**1**) would possess two *trans*-fused rings with 5α-axial

Table 1. The ^{1}H NMR spectra of rishitinone (**1**) in the presence of the shift reagent $Eu(dpm)_3$ (CCl_4, 100 MHz) and the results of the spin-decoupling studies

Protons irradiated (δ and Hz)	Observed multiplicity change (δ and Hz)
Mole ratio of **1**: $Eu(dpm)_3$ 5 : 1	
6.80 [Ha (C-2), br (W_H=25)	4.00[Hb, dt → dd(J=12 and 4)]
	3.20—3.80[Hc—He, br m → changed]
4.00[Hb(C-1), dt(J=12 and 4)]	6.80[Ha, br → changed]
3.50[Hc—He(C-1—C-3), br m]	6.80[Ha, br → changed]
	2.30[Hf, br → changed]
2.34[Hf(C-4), br]	1.23[CH_3, d → s]
1.23[CH_3(C-4), d(J=7)]	2.30[Hf, br → dd(J=11 and 3)]
Mole ratio of **1**: Eu $(dpm)_3$ 5 : 2	
10.5[Ha(C-2), br(W_H=25)]	5.6—6.4[Hb—He, br m → changed]
6.12[Hb and Hc(C-1), br m]	4.12[Hg, dd → s]
4.12[Hg(C-10), dd(J=12 and 4)]	6.0—6.3[Hb—Hc, br m → changed]



4 **5**



6 **7**

methyl group and 10β-hydrogen atom was deduced from the following facts: (i) dihydrorishitinone (**2**) showed a typical positive Cotton effect with a=+25.7, and the value was in good accord with that reported[6] (a=+26) for ketone **4**, which had been derived from natural (−)-aristolochene[6] (**5**), a valencane sesquiterpene with the established configurations. (ii) Angular methyl protons of *trans*-10-methyl-1-decalones (**6**) are usually observed at higher fields than the corresponding protons of the *cis*-analogues (**7**): *trans*, δ 0.7—0.9; *cis*, δ 1.0—1.2.[7] The observed chemical shifts (δ 0.76, 0.73, and 0.67) of **1**, **1a**, and **2** were in good agreement with those of the *trans*-fused model compounds (**6**).

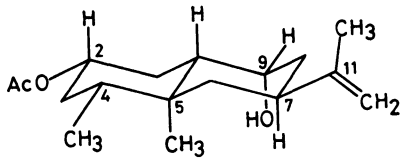
The hydroxyl at C-2 and methyl groups at C-4 were assigned equatorial conformation and hence α-configuration on the basis of the spectral data. The signal multiplicities in Table 1 (*cf.*, formula A) revealed the following coupling constants: $J_{g\text{-}b}$, $J_{g\text{-}c}$, $J_{a\text{-}b}$, $J_{b\text{-}c}$, and $J_{f\text{-}e}$ (or $J_{f\text{-}d}$)=4, 12, 4, 12, and 11 Hz, respectively. Since the ring A must take a chair conformation, these values, combined with the large half-width value (25 Hz) of the signal due to the C-2 proton (Ha), not only support the axially oriented β-configuration of the C-10 hydrogen atom but also confirm the afore-mentioned stereochemical assignment to the relevant groups at C-2 and C-4.

The presumption that the isopropenyl group would be located at C-7 and possess β-configuration was deduced from interpretation of the ^{1}H NMR spectrum of the hydroxy acetate (**3**), in which two new well-resolved signals were observed at δ 3.95 (1H, q, J=4 Hz) and 2.52 (1H, tt, J=12 and 4 Hz). The former was ascribed readily to the hydroxy-methine proton at C-9, and its coupling constants indicated that the hydrogen is oriented equatorial (β-configuration). On the other hand, the latter would be attributed reasonably to the proton on the carbon adjacent to the isopropenyl group. The observed multiplicity accounting for two axial-axial (J=12 Hz) and two equatorial-axial (J=4 Hz) coupling constants indicated that the carbon atom in question must be flanked by two unsubstituted methylene groups, and hence the isopropenyl group must be disposed at C-7 (neither C-6 nor C-8). Moreover, the splitting pattern revealed that the relevant C-7 proton is oriented axial, and hence the isopropenyl group must take β-configuration. It follows that the angular methyl group at C-5, the hydrogen atom at C-7, and the hydroxyl group at C-9 all have 1,3-diaxial relationship each other on the ring B with a chair conformation. In accordance with this conformational assignment, the hydroxy acetate (**3**) exhibited the signal due to the C-5 angular methyl protons at lower field (δ 1.02) than the corresponding oxo acetate (**1a**) (δ 0.73).[8] Furthermore, in the ^{1}H NMR spectrum in pyridine-d_5, remarkable down-field shifts were observed for the C-7 proton ($\Delta\delta$ 0.35) and the C-5 angular methyl protons ($\Delta\delta$ 0.25) (Table 2) (the solvent-induced NMR shift correlation).[9] In summary, all the chemical evidence and spectral data strongly suggested that rishitinone would probably be represented by formula **1**. This presumption was confirmed by correlation of **1** with (+)-nootkatone[10] (**8**), a well-known sesquiterpene with the established configurations, as described below.

Transformation of Nootkatone into Dihydrorishitinone. (+)-Nootkatone[10] (**8**), mp 28—30 °C, $[\alpha]_D$ +172°, was submitted to partial hydrogenation in benzene, containing tris(triphenylphosphine)chlororhodium[11] at room temperature for 14 h to give dihydronootkatone (**9**), oil, $[\alpha]_D$ +167°, in 81% yield. The compound (**9**) was converted by a modification of the Edwards procedure[12] into the corresponding dienol acetate (**10**), oil, $[\alpha]_D$ +45.3°, in 69% yield, showing the following

TABLE 2. THE SOLVENT EFFECT IN THE CHEMICAL SHIFTS OF HYDROXY ACETATE (3) IN CHLOROFORM-*d* AND PYRIDINE-d_5

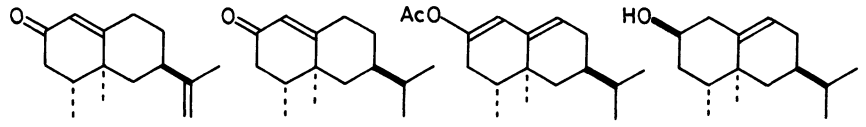
Proton	Chemical shift δ		
	$CDCl_3$	C_5D_5N	$\Delta\delta$
14-H (4-CH_3)	0.79 (3H, d, J=6 Hz)	0.76	−0.03
15-H (5-CH_3)	1.02 (3H, s)	1.27	0.25
13-H (11-CH_3)	1.72 (3H, s)	1.76	0.04
$OCOCH_3$	2.02 (3H, s)	2.04	0.02
7-H	2.52 (1H, tt, J=12 and 4 Hz)	2.87	0.35
9-H	3.95 (1H,q, J=4 Hz)	4.05	0.10
12-H(11-CH_2)	4.75 (2H, s)	4.87	0.12
2-H	4.77 (1H, m, W=25 Hz)	4.96	0.10



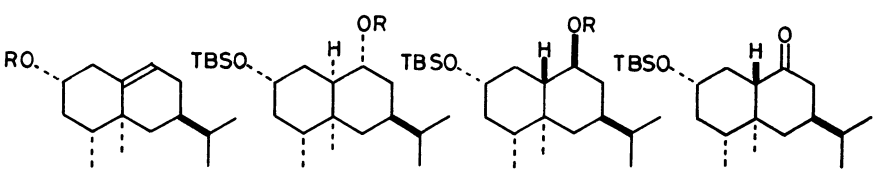
3

spectra: IR, 1759 and 1664 cm^{-1}; NMR, δ 2.12 (3H, s, $OCOCH_3$), 5.42 (1H, W_H=10 Hz, 9-H), and 5.71 (1H, s, 1-H). Reduction of **10** with sodium borohydride produced a 1 : 5 mixture of stereoisomeric homoallyl alcohols (**11**) and (**12**), which were separated by preparative TLC. The minor (**11**) (9%), oil, $[\alpha]_D$ −30.7°, and the major alcohols (**12**) (45%), oil, $[\alpha]_D$ −34.2°, were assigned 2-axial and 2-equatorial alcohols respectively, on the basis of the NMR spectra: **11**, δ 3.99 (1H, m, W_H=8 Hz, 2-H) and 5.43 (1H, m, W_H= 12 Hz, 9-H); **12**, δ 3.56 (1H, m, W_H=25 Hz, 2-H) and 5.38 (1H, m, W_H=12 Hz, 9-H). The hydroxyl group of the latter (**12**) was then protected by treatment with *t*-butyldimethylsilyl (TBS) chloride and imidazole in *N,N*-dimethylformamide. The resulting silyl ether (**12a**), oil, $[\alpha]_D$ −16.3°, obtained in 76% yield, underwent hydroboration followed by oxidation with alkaline hydrogen peroxide, producing a mixture of the starting alkene (**12a**) and two stereoisomeric alcohols (**13**) and (**14**), which were separated by column chromatography in 23, 8 (crude), and 42% (pure) yields, respectively. The major product (**14**), oil, $[\alpha]_D$ +38.0°, was assigned 9β-hydroxy-*trans*-valencane structure on the basis of the spectral data: MS, *m/e* 354 (M^+); IR, 3360 cm^{-1}; NMR, δ 0.72 (3H, s, 15-H), 3.49 (1H, dt, J=4.5 and 10 Hz, 9-H), and 3.58 (1H, m, W_H=25 Hz, 2-H). Supporting this assignment, the acetate (**14a**), oil, $[\alpha]_D$ +35.5°, of **14** displayed the following NMR spectrum: δ 0.76 (3H, s, 15-H), 3.76 (1H, m, W_H=25 Hz, 2-H), and 4.74 (1H, dt, J=4 and 11 Hz, 9-H). The assigned stereostructure to the major alcohol (**14**) was also consistent with the currently accepted steric manner of hydroboration; namely, the reaction of the alkene (**12a**) took place mainly in a *cis*-addition manner at the less-hindered β-side opposite to the angular methyl group,[6,13] in contrast with formation of *cis*-eremophilane from dihydroeremophilene.[14] On the other hand, the minor crude alcohol (**13**) was submitted to acetylation followed by purification by chromatography. The resultant pure acetate (**13a**), oil, $[\alpha]_D$ −2.8°, exhibited two signals due to the C-2 and C-9 protons at δ 3.60 (1H, m, W_H=25 Hz) and 5.00 (1H, q, J=4 Hz), respectively, in the NMR spectrum, indicative of the axial conformation of the relevant hydroxyl group at C-9.

The *trans*-decalol (**14**) was oxidized with Collins reagent at room temperature for 15 h to yield the corresponding ketone (**15**), mp 51—53 °C, $[\alpha]_D$ +2.1°, in a quantitative yield, showing the IR absorption at 1717 cm^{-1}. Hydrolysis of the silyloxy ketone (**15**)

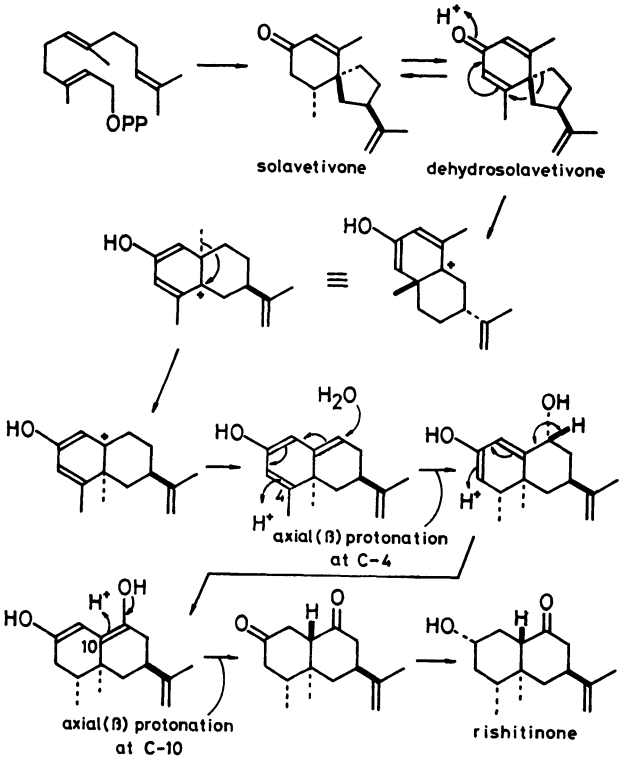


8 9 10 11



12 R = H
12a R = TBS
13 R = H
13a R = Ac
14 R = H
14a R = Ac
15

TBS = t-Bu$(CH_3)_2$Si



Scheme 1.

without epimerization at C-10 was effected by treatment with a 3 : 1 : 1 mixture of acetic acid, water, and tetrahydrofuran to give hydroxy ketone, mp 82—83 °C, $[\alpha]_D$ +7.8°, in 95% yield, which was identical with an authentic specimen of natural dihydrorishitinone (**2**) in all respects. All these results establish that the structure and configuration of (+)-rishitinone are represented by formula **1**.

Rishitinone (**1**), a stress metabolite with the valencane skeletone from diseased potato tubers, has a comparable fungitoxicity as rishitin, and would be qualified as a phytoalexin. In view of the facts that the phytoalexins (stress metabolites) produced in the Solanaceae plants possess structures which differ from usual sesquiterpenes in planar structure and/or configuration,[3] the following scheme 1 is proposed as one of plausible biogeneses for formation of the compound (**1**), considering the biosynthetic pathway of rishitin.[15]

Experimental

All the melting points were uncorrected. The purity of each compound was always checked by TLC over silica gel (Wakogel B-5 or Merck GF-254) with various solvent systems, and the spots were developed with cerium(IV) sulfate in dil sulfuric acid, iodine, and/or concd sulfuric acid. The optical rotations, UV, IR, and NMR (100 MHz) were measured in ethanol, in ethanol, in liquid state (oil) or in Nujol (crystals), and in chloroform-*d*, respectively, unless otherwise stated. The preparative TLC was carried out over silica gel (Merck GF-254), and the column chromatography over silica gel (Mallinckrodt AR-100) or silicic acid (Kiesel gel 60), respectively.

Isolation of Rishitinone (1). The neutral chloroform extracts[4] (83 g), obtained from the diseased potato tubers[4] (300 kg) were separated roughly into five fractions I—V by column chromatography over silica gel (1.8 kg), benzene, benzene–ethyl acetate (1 : 1), ether, ethyl acetate, and methanol being used as eluents, successively. Fraction III (5.5 g), yellow oil, corresponding to "fraction F,"[4] was treated with acetic anhydride and pyridine, and the acetate mixture was separated by column chromatography over silica gel to give a semi-crystalline mixture (3.4 g), composing of rishitin diacetate[4] and rishitinone acetate (**1a**). Fractional recrystallization of the mixture from hexane afforded pure rishitin diacetate (3.3 g), mp 65—67 °C, and crude **1a** (112 mg). The latter was again purified by chromatography over silica gel (5.0 g) with a 10 : 1 mixture of hexane–ethyl acetate as an eluent to yield a crude semi-crystalline acetate mixture (82 mg), which was subjected to repeated crystallization from hexane, the resulting crystals being removed by filtration. Pure rishitinone acetate (**1a**) (68 mg) was obtained from the filtrate as an oil, $[\alpha]_D$ −2.4°, which on hydrolysis with potassium carbonate in methanol at room temperature for 1 h under nitrogen gave rishitinone (**1**), mp 72—75 °C (from diisopropyl ether), $[\alpha]_D$ +10.1°; MS, *m/e* 236 (M^+), 221, 218, 203, 193, and 153 (base); IR, 3500, 3400, 1710, 1651, and 900 cm^{-1}; ^1H NMR, δ 0.76 (3H, s, 15-H), 0.84 (3H, d, J=7 Hz, 14-H), 1.76 (3H, s, 13-H), 3.65 (1H, m, W_H=25 Hz, 2-H), and 4.77 (2H, br s, 12-H); ^{13}C NMR, δ 12.9, 15.5, and 20.4 (each q), 30.2, 39.3, 42.8, 46.0, and 109.9 (each t), 41.3, 41.5, 56.1, and 69.5 (each d), 40.7, 147.4, and 211.1 (each s). Found: C, 76.38; H, 10.01%. Calcd for $C_{15}H_{24}O_2$: C, 76.22; H, 10.24%.

Acetylation and Hydrogenation of 1. Compound **1** (14 mg) was treated with acetic anhydride (0.2 ml) and pyridine (1 ml) at room temperature for 6 h. The reaction mixture was worked up as usual to give rishitinone acetate (**1a**) (16 mg), oil, $[\alpha]_D$ −2.4°; MS, *m/e* 278 (M^+), 236, 218, 203 ,195, and 135 (base); IR, 1739, 1717, 1644, 1248, 1030, and 893 cm^{-1}; ^1H NMR, δ 0.73 (3H, s, 15-H), 0.89 (3H, d, J=6 Hz, 14-H), 1.75 (3H, s, 13-H), 2.02 (3H, s, $OCOCH_3$), 4.77 (1H, m, W_H= 25 Hz, 2-H), and 4.80 (2H, s, 12-H).

A solution of **1** (13 mg) in ethanol (1.0 ml) was hydrogenated over 5% palladized charcoal (1.5 mg) at room temperature for 20 h under stirring. After removal of the catalyst the reaction mixture was evaporated *in vacuo* to leave a colorless crystalline substance, which was recrystallized from diisopropyl ether to afford dihydrorishitinone (**2**) (12 mg), mp 80—82 °C, $[\alpha]_D$ +7.2°; ORD, $[\Phi]_{310}^{peak}$ +1140°, $[\Phi]_{268}^{trough}$ −1430°, a= 25.7; MS, *m/e* 238 (M^+), 223, 205, 195, 177, 166, 153, 140, and 97; IR, 3580, 3440, 1702, 1388, 1372, 1063, and 1027 cm^{-1}; ^1H NMR, δ 0.67 (3H, s, 15-H), 0.90 (3H, d, J=6 Hz, 14-H), 0.91 (6H, d, J=6 Hz, 12- and 13-H), and 3.61 (1H, m, W_H= 25 Hz, 2-H).

Reduction of 1a with Sodium Borohydride ($NaBH_4$). A solution of **1a** (12 mg) in ethanol (1.5 ml) was stirred with $NaBH_4$ (20 mg) at room temperature for 30 min under nitrogen. The reaction mixture was diluted with ether (30 ml), and the whole mixture was washed with water (10 ml) and saturated brine (10 ml), dried, and evaporated to leave a colorless oil (12 mg), showing a single spot on TLC, which crystallized on trituration with hexane. Recrystallization from hexane–diisopropyl ether afforded hydroxy acetate (**3**) (10 mg), mp 96—98 °C, $[\alpha]_D$ −6.9°; MS, *m/e* 280 (M^+), 262, and 220; IR, 3620, 3480, 1728, 1645, 1259, 1028, and 895 cm^{-1}; ^1H NMR in $CDCl_3$ and C_5D_5N, in Table 2.

Hydrogenation of Nootkatone (8). A solution of **8** (653 mg), mp 28—30 °C, $[\alpha]_D$ +172° (lit,[10] mp 28—30 °C, $[\alpha]_D$ +170°), and tris(triphenylphosphine)chlororhodium (450 mg) in benzene (90 ml) was stirred with hydrogen at room temperature for 14 h, and the mixture was passed through an activated alumina column (Merck, activity II—III, 30 g). The column

was eluted with benzene–ethyl acetate (1 : 1) (0.5 l), and the eluate was evaporated to give dihydronootkatone (**9**) (523 mg), showing a single spot on TLC, colorless oil, $[\alpha]_D$ +167°; MS, *m/e* 220 (M^+), 178, 177, and 135 (base); IR, 1670, 1621, 1435, 1414, 1383, 1372, 1355, and 1290 cm^{-1}; ^1H NMR, δ 0.69, 0.72, and 0.78 (each 3H, d, J=7 Hz, 12-, 13-, and 14-H, or *vice versa*), 1.08 (3H, s, 15-H), and 5.78 (1H, s, 1-H). Found: C, 81.45; H, 11.23%. Calcd for $C_{15}H_{24}O$: C, 81.76; H, 10.16%.

Treatment of **9** *with Acetic Anhydride and Perchloric Acid.* The Edwards reagent A,[12] an absolute ethyl acetate solution (50 ml) containing acetic anhydride (4.8 ml) and 60% perchloric acid (0.06 ml), was prepared according to the reported procedure. Compound **9** (350 mg) was dissolved in the soltion and stirred at room temperature for 10 min. The reaction mixture was mixed with 5% aqueous sodiumhydrogencarbonate (20 ml), shaken, and separated. The aqueous solution was extracted with ethyl acetate (2 × 10 ml). The combined acetate solution was washed with 2 M hydrochloric acid (10 ml), 5% aqueous sodium hydrogencarbonate (10 ml), and saturated brine (10 ml), dried, and evaporated to yield an oily substance (362 mg), which was purified by column chromatography over silica gel (15 g). Elution with hexane–ethyl acetate (20 : 1) afforded dienol acetate (**10**) (353 mg), showing a single spot on TLC, oil, $[\alpha]_D$ +45.3°; MS, *m/e* 262 (M^+), 236, 220, and 177; IR, 1759, 1664, and 1212 cm^{-1}; ^1H NMR, δ 0.90 (12H, br s, 12-, 13-, 14-, and 15-H), 2.12 (3H, s, $OCOCH_3$), 5.42 (1H, m, W_H=10 Hz, 9-H), and 5.71 (1H, s, 1-H).

Reduction of **10** *with* $NaBH_4$. A solution of **10** (300 mg) in ethanol (20 ml) was stirred with $NaBH_4$ (300 mg) at room temperature for 3 h. The reaction mixture was evaporated *in vacuo*, mixed with water (30 ml), and extracted with ether (50 ml). The ether solution was worked up as usual to leave an oily substance, consisting of two components on TLC, which were successively separated by column chromatography over silica gel (20 g) with hexane–ethyl acetate (5 : 1) as an eluent to give two epimeric homoallyl alcohols (**11**) (22 mg) and (**12**) (118 mg): **11**, oil, $[\alpha]_D$ −30.7°; MS, *m/e* 222 (M^+), 204, 189, 175, and 161; IR, 3300, 1382, 1365, 1060, 1024, and 1002 cm^{-1}; ^1H NMR, δ 0.90 (12H, br s, 12-, 13-, 14-, and 15-H), 2.58 (1H, dt, J=14 and 2 Hz, 1-H), 3.99 (1H, m, W_H=8 Hz, 2-H), and 5.43 (1H, m, W_H=12 Hz, 9-H): **12**, oil, $[\alpha]_D$ −34.2°; MS, *m/e* 222 (M^+), 204, 189, 179, and 161; IR, 3320, 1381, 1368, and 1021 cm^{-1}; ^1H NMR, δ 0.90 (12H, br s, 12-, 13-, 14-, and 15-H), 3.56 (1H, m, W_H=25 Hz, 2-H), and 5.38 (1H, m, W_H=12 Hz).

t-*Butyldimethylsilylation of* **12**. A solution of **12** (104 mg) in *N*,*N*-dimethylformamide (4 ml) was stirred with *t*-butyldimethylsilyl (TBS) chloride (200 mg) and imidazole (180 mg) at room temperature for 8 h. The reaction mixture was cooled, diluted with water (30 ml), and extracted with ether (4 × 20 ml). The combined ether solutions were washed with water (2 × 30 ml), dried, and evaporated to give practically pure silyl ether (**12a**) (120 mg), showing a single spot on TLC, oil, $[\alpha]_D$ −16.3°; MS, *m/e* 337 (M^+ +1), 279, 203, and 147 (base); IR, 1460, 1370, 1258, 1100, 1060, 910, 883, 850, and 839; ^1H NMR, δ 0.90 (12H, br s, 12-, 13-, 14- and 15-H), 3.49 (1H, m, W_H=25 Hz, 2-H), and 5.35 (1H, m, W_H=12 Hz, 9-H).

Hydroboration of **12a** *Followed by Oxidation.* To a cooled solution of **12a** (110 mg) in tetrahydrofuran (THF) (0.5 ml) at 0 °C was added a 1.87 M solution of diborane in THF (0.5 ml). The mixture was stirred at room temperature for 1 h and cooled at 0 °C, and water (2 ml) was then added to decompose the excess reagent. The aqueous mixture was stirred with 3 M aqueous sodium hydroxide (0.1 ml) and 30% hydrogen peroxide (0.2 ml) at 0 °C for 1 h. The reaction mixture was diluted with water (20 ml) and extracted with ether (5 × 10 ml). The combined ether solution was worked up as usual to leave an oily residue (113 mg), which was separated into three fractions by column chromatography over silica gel with hexane–ethyl acetate (1 : 1) as an eluent. The most mobile fraction (25 mg) gave the practically pure starting alkene (**12a**). The middle fraction (8 mg) showed three spots on TLC and was then treated with acetic anhydride (0.1 ml) and pyridine (1 ml) at room temperature overnight for purification. The reaction mixture was purified by preparative TLC over silica gel with hexane–ethyl acetate (10 : 1) as a solvent. A fraction with R_f value of 0.36 afforded 9α-acetate (**13a**) (4 mg) in pure state, oil, $[\alpha]_D$ −2.8°; MS, *m/e* 395 (M^+ −1), 339, 335, 279, and 205; IR, 1721, 1252, 1100, 1051, 1020, 855, and 836 cm^{-1}; ^1H NMR, δ 0.05 (6H, s), 0.88 (9H, s), and 0.90 (br s, 12-, 13-, 14-, and 15-H), 2.04 (3H, s, $OCOCH_3$), 3.60 (1H, m, W_H=25 Hz), and 5.00 (1H, q, J=4 Hz, 9-H). The least mobile fraction (49 mg), showing a single spot on TLC, was purified by preparative TLC to give 9β-alcohol (**14**), oil, $[\alpha]_D$ +38.0°; MS, *m/e* 354 (M^+), 229, 205, and 75 (base); IR, 3360, 1258, 1100, 1077, 1050, 1038, 859, 837, and 773 cm^{-1}; ^1H NMR, δ 0.05 (6H, s), 0.72 (3H, s, 15-H), 0.88 (9H, s), 0.90 (9H, br s, 12-, 13-, and 14-H), 3.49 (1H, dt, J=4.5 and 10 Hz, 9-H), and 3.58 (1H, m, W_H= 25 Hz, 2-H). Compound **14** (49 mg) was converted by treatment with acetic anhydride (0.5 ml) and pyridine (2 ml) at room temperature for 14 h into the corresponding 9β-acetate (**14a**) (52 mg), oil, $[\alpha]_D$ +35.5°; MS, *m/e* 395 (M^+ −1), 335, 279, and 205 (base); IR, 1740, 1240, 1238, 1100, 1077, 1053, 1030, 856, 833, and 770 cm^{-1}; ^1H NMR, δ 0.05 (6H, s), 0.76 (3H, s, 15-H), 0.88 (9H, s), 0.90 (9H, br s, 12-, 13-, and 14-H), 2.02 (3H, s, $OCOCH_3$), 3.76 (1H, m, W_H= 25 Hz, 2-H), and 4.74 (1H, dt, J=4 and 11 Hz).

Collins Oxidation of **14**. A mixture of chromium(VI) oxide (54 mg) and pyridine (54 μl) in dichloromethane (0.8 ml) was stirred at room temperature for 15 min under nitrogen. To the mixture was added a solution of **14** (16 mg) in dichloromethane (0.2 ml) under stirring. The mixture was further stirred at room temperature for 15 h, and diluted with ether (40 ml) and filtered. The filtrate was washed with 5% aqueous sodium hydrogencarbonate (2 × 30 ml), dried, and evaporated to yield a crystalline substance, which on recrystallization from diisopropyl ether gave ketone (**15**) (15 mg), mp 51—53 °C, $[\alpha]_D$ +2.1°; MS, *m/e* 352 (M^+), 337, 309, and 295 (base); IR, 1717, 1468, 1390, 1380, 1372, 1362, 1258, 1101, 1080, 1057, 860, 839, and 778 cm^{-1}; ^1H NMR, δ 0.05 (6H, s), 0.66 (3H, s, 15-H), 0.88 (9H, s), 0.90 (9H, br s, 12-, 13-, and 14-H), 3.78 (1H, m, W_H=25 Hz, 2-H).

Hydrolysis of **15**. A solution of **15** (14 mg) in a 3 : 1 : 1 mixture (2 ml) of acetic acid, water, and THF was stirred at room temperature for 17 h. The reaction mixture was diluted with ether (40 ml) and washed with 5% aqueous sodium hydrogencarbonate (15 ml), dried, and evaporated to leave a crystalline substance, which on recrystallization from diisopropyl ether afforded hydroxy ketone (7 mg), mp 82—83 °C, $[\alpha]_D$ +7.8°; MS, *m/e* 238 (M^+), 223, 220, 205, 195, 177, 168, 167, 166, 153, 140, and 97; IR, 3580, 3440, 1702 1063, and 1027 cm^{-1}; ^1H NMR, δ 0.66 (3H, s, 15-H), 0.89 (6H, d, J=6 Hz, 12- and 13-H), 0.91 (3H, d, J=6 Hz, 14-H), and 3.62 (1H, m, W_H=25 Hz, 2-H). The compound was identical with an authentic sampel of dihydrorishitinone (**2**) derived from natural rishitinone (**1**) in all respects (TLC, $[\alpha]_D$, MS, IR, ^1H NMR, and mixed mp).

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