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BIOMIMETIC SYNTHESIS OF THE IRIDAL SKELETON

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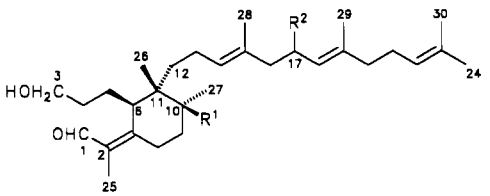
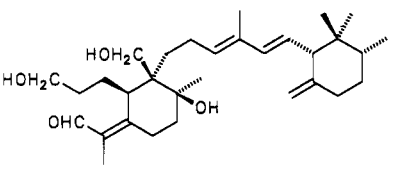
ABSTRACT.—A biomimetic synthesis of the iridal skeleton, giving access to possible biogenetic intermediates of these triterpenoids from plants in the family Iridaceae or of the related triterpenoid, crystallopicrin, which has been isolated from various *Cortinarius* species, is described.

The iridals are a family of unusual monocyclic, bicyclic, or spirocyclic triterpenoids (e.g., **1**–**4**), which are found in the lipid-soluble extracts of species of the family Iridaceae (1–3). Cyclization of 2,3-epoxysqualene leads to the formation of a bicyclic intermediate, which is subsequently rearranged to give the characteristic seco-ring A structure of these natural products (1). In the course of this reaction, a CH_3 group is shifted from C-6 to C-11[1], and the first product to be expected is the aldehyde **5**, not as yet detected in Nature (Scheme 1). The stereochemistry at C-10 depends on the folding of the epoxysqualene during its cyclization. The evidence favors a chair-boat form, necessitating a 6*S*,10*R*,11*R* geometry of the product, which has been found for 10-deoxy-17-hydroxyiridal [**2**], isolated from *Iris sibirica* (3). Isomer **5a** with opposite stereochemistry at C-10 should result from the reaction of the epoxide, folded in a chair-chair geometry. The relative configuration of **5a** has been found for crystallopicrin [**6**], the only other triterpenoid obtained to date with an iridal skeleton, which was discovered in several *Cortinarius* species (4).

Our interest in the biosynthesis of these natural products led us to look for a synthesis of possible biogenetic precursors, which may be useful in incorporation studies or as reference compounds



1 R^1=OH, R^2=H
2 R^1=H, R^2=OH



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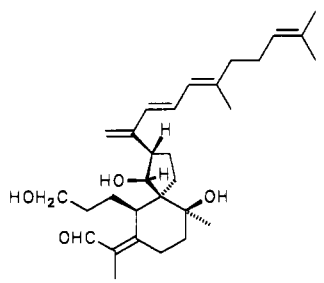

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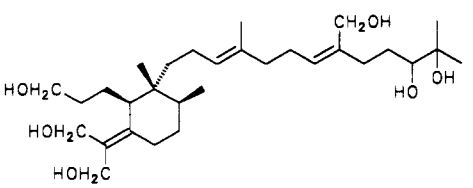

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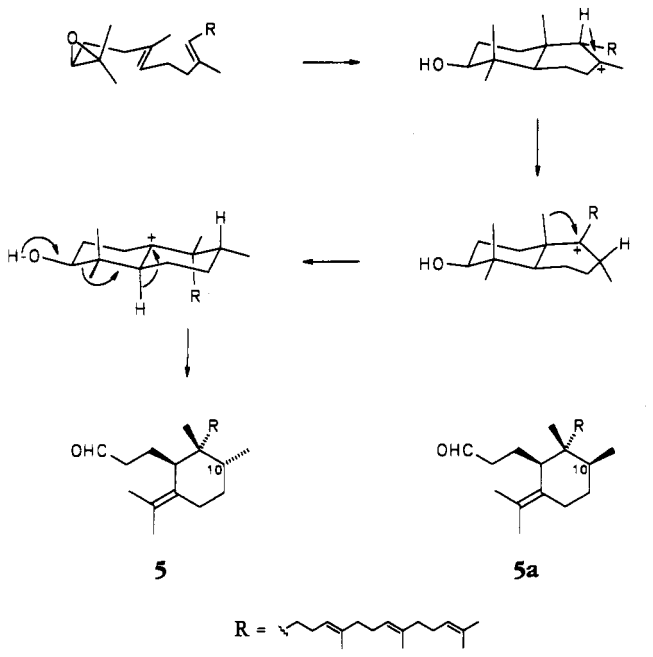
[1]The carbon skeleton of all compounds has been numbered by analogy to squalene.

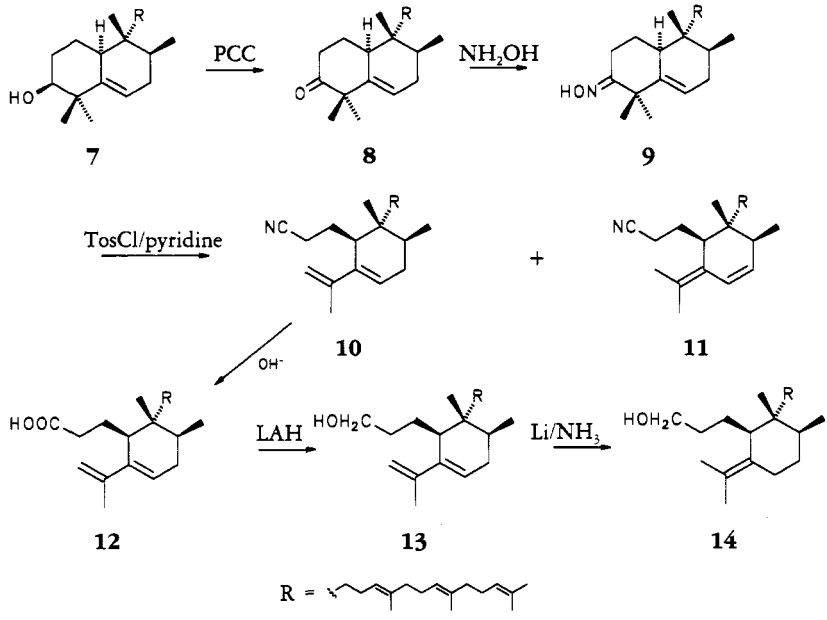


SCHEME 1. Proposed biosynthesis of the iridals.

for the detection of hitherto unknown intermediates. The synthetic pathway is depicted in Scheme 2.

The bicyclic alcohol **7**, which is obtained *inter alia* as an enantiomeric mixture by van Tamelen's biomimetic cyclization of 2,3-epoxysqualene (5), was chosen as starting material, since ring B should show the correct substitution pattern with the relative configuration of crystallopicrin [**6**] after cleavage of ring A between C-2 and C-3. A minor drawback was the very complex mixture arising from the cyclization of the epoxide with $SnCl_4$. Thus, the isolation of preparative amounts of **7** was rather tedious. However, the alcohol can be enriched by mplc (RP-18) to approximately 60% purity and final separation was achieved by chromatography on $AgNO_3$-impregnated Si gel. Oxidation of the alcohol with pyridine chlorochromate (PCC) gave the ketone **8**, which was easily purified on Si gel. Because even remaining side products of the cyclization are removed this way, the partial purification of **7** by reversed-phase chromatography was sufficient. For the opening of ring A the procedure outlined by Whitham was used for the synthesis of nyctanthic acid (6). Ketone **8** was converted to the oxime **9**. Treatment of the crude product with *p*-toluene sulfonyl chloride in pyridine gave a 1:1 mixture of the two isomeric nitriles **10** and **11** in moderate yield. The isomers were separated on Si gel. The rather unstable carboxylic acid **12** was obtained by alkaline hydrolysis of nitrile **10**. Therefore, the crude product was immediately reduced to give the alcohol **13**. Finally, Birch reduction of the conjugated diene system with Li/NH_3 afforded the alcohol **14** with an iridal skeleton (Scheme 2).

A reference sample of the aldehyde **5a** was obtained by oxidation of a small amount of **14** with CrO_3/pyridine. However, no components with comparable ms data were found in lipid-soluble extracts of various *Iris* species nor in cell suspension cultures of *I. pallida* or *I. pseudacorus*, respectively. Presumably, the intermediates of the proposed biosynthesis are not stored in detectable amounts. Experiments to promote the accumulation of



SCHEME 2. Synthesis of the seco-ring A alcohol **14**.

these compounds by incubation of the *Iris* cell suspension cultures with appropriate enzyme inhibitors are underway.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Gc: Shimadzu GC-8A gas chromatograph, column, OV-101 (15 m×0.25 mm i.d.); carrier gas, H_2 (2 ml/min). Cc: Merck Kieselgel 60 (63–200 μm). Mplc: Büchi model 681; column, RP-18 14-40 μm (240 mm, 20 mm i.d.). Nmr spectra: Bruker AC 80 E (^{1}H: 80 MHz) and Bruker WH 300 (^{1}H: 300 MHz, ^{13}C: 75 MHz) spectrometers in $CDCl_3$. Assignments are based on comparison with Refs. (1–3, 7). Eims: Finnigan MAT 4510 GCMS (70 eV). All reactions were run in oven-dried glassware under Ar. Commercially available chemicals were used without further purification. Solvents were distilled, and if necessary, dried by standard methods prior to use.

Bicyclic alcohol [**7**].—2,3-Epoxysqualene (2.75 g, 6.5 mmol) was cyclized with $SnCl_4$ according to Sharpless and van Tamelen (5). The crude product was purified by mplc with MeOH-H_2O (90:10) as eluent to afford 634 mg (23%) of the bicyclic alcohol **7** in 60% purity (gc) as a colorless oil. For its spectroscopic characterization, further purification of 100 mg of **7** was carried out by chromatography on $AgNO_3$-impregnated Si gel (8) with pentane-Et_2O (70:30) as eluent, to give 12 mg of pure **7**. ^{1}H nmr (80 MHz) δ 5.42 (1H, m, H-8), 5.1 (3H, m, H-14, H-18, H-22), 3.44 (1H, m, H-3), 2.0 (20H, m), 1.67 (3H, s, H_3-24), 1.59 (9H, s, H_3-28, H_3-29, H_3-30), 1.1 (6H, s, H_3-1, H_3-25), 0.84 (3H, s, H_3-26), 0.7 (3H, d, J=6 Hz, H_3-27); eims *m/z* $[M]^+$ 426 (0.2), 408 (0.1), 271 (0.2), 215 (0.4), 135 (62), 119 (15), 107 (21), 95 (25), 81 (42), 69 (100), 55 (26), 41 (55).

Bicyclic ketone [**8**].—PCC (750 mg) was added to a solution of 634 mg of bicyclic alcohol **7** (60%, 1.5 mmol) in 15 ml of CH_2Cl_2. The mixture was stirred at room temperature for 5 h, the solvent was removed *in vacuo*, and the black residue was extracted three times with 10 ml of pentane-Et_2O (1:1). The combined organic phases were washed with H_2O, dried ($MgSO_4$), and evaporated to yield a yellow oil, which was chromatographed on Si gel (petroleum ether-Et_2O, 97:3) to afford the bicyclic ketone **8** (448 mg, 70%) as a colorless oil. ^{1}H nmr (300 MHz) δ 5.47 (1H, t, J=3.3 Hz, H-8), 5.08 (3H, m, H-14, H-18, H-22), 2.65–2.25 (2H, m, H-4), 2.2–1.85 (18H, m), 1.66 (3H, s, H_3-24), 1.57 (9H, s, H_3-28, H_3-29, H_3-30), 1.23 (3H, s, H_3-1 or H_3-25), 1.18 (3H, s, H_3-1 or H_3-25), 0.86 (3H, s, H_3-26), 0.82 (3H, d, J=6.6 Hz, H_3-27); ^{13}C nmr (75 MHz) δ 215.6 (s, C-3), 143.3 (s, C-7), 134.9 (s, C-15), 134.7 (s, C-19), 131.3 (s, C-23), 125.0 (d, C-22), 124.4 (d, C-18), 124.2 (d, C-14), 118.8 (d, C-8), 51.7 (s, C-2), 40.0 (d, C-6), 39.7 (2C, t, C-16, C-20), 37.9 (t, C-4), 36.1 (s, C-11), 32.5 (d, C-10), 31.3 (t, C-9), 27.1 (q), 26.7 (t, C-21), 26.6 (t, C-17), 25.9 (t), 25.7 (q, C-24), 22.2 (q), 22.0 (q), 21.9 (t), 17.7 (q, C-30), 16.0 (q, C-29), 15.9 (q, C-28), 14.6 (q, C-27); eims *m/z* $[M]^+$ 424 (1.2), 381 (0.2), 287 (0.5), 245 (0.5), 231 (1), 218 (1.5), 205 (24), 163 (9), 147 (7), 136 (17), 119 (12), 107 (15), 95 (26), 81 (48), 69 (100), 55 (22), 41 (46).

Bicyclic oxime [**9**].—Hydroxylamine hydrochloride (150 mg, 2.2 mmol) and NaOAc (300 mg, 2.2 mmol) were added to a solution of 300 mg (0.71 mmol) of the bicyclic ketone **8** in 60 ml EtOH, and the mixture was stirred for 6 h at room temperature. The solvent was removed *in vacuo* and the residue was dissolved in Et_2O. The solution was washed with H_2O, dried ($MgSO_4$), and evaporated to yield the crude oxime **9** (287 mg, 92%) as a colorless oil, which was used without further purification. For the spectroscopic characterization the compound was isolated by chromatography on deactivated (10% H_2O) Si gel (petroleum ether-Et_2O, 95:5). ^{1}H nmr (300 MHz) δ 5.48 (1H, t, J=3.3 Hz, H-8), 5.09 (3H, m, H-14, H-18, H-22), 2.2–1.85 (20H, m), 1.66 (3H, s, H_3-24), 1.58 (9H, s, H_3-28, H_3-29, H_3-30), 1.28 (3H, s, H_3-1 or H_3-25), 1.25 (3H, s, H_3-1 or H_3-25), 0.83 (3H, s, H_3-26), 0.79 (3H, d, J=6.6 Hz, H_3-27); ^{13}C nmr (75 MHz) δ 166.0 (s, C-3), 144.1 (s, C-7), 134.9 (s, C-15), 134.5 (s, C-19), 131.2 (s, C-23), 125.1 (d, C-22), 124.4 (d, C-18), 124.2 (d, C-14), 117.4 (d, C-8), 43.6 (s, C-2), 40.3 (d, C-6), 39.7 (2C, t, C-16, C-20), 36.0 (s, C-11), 32.5 (d, C-10), 31.1 (t, C-9), 28.2 (t), 28.0 (t), 26.7 (t, C-21), 26.6 (2C, t), 25.7 (q, C-24), 24.7 (q), 21.9 (q), 21.8 (t), 21.1 (q), 17.7 (q, C-30), 16.0 (q, C-29), 15.9 (q, C-28), 14.6 (q, C-27); eims m/z $[M]^+$ 439 (1.7), 422 (1.3), 302 (0.5), 220 (11), 204 (4), 160 (5), 136 (6), 121 (7), 107 (8), 95 (14), 81 (33), 69 (100), 55 (17), 41 (51).

Monocyclic nitriles [**10** and **11**].—A solution of 190 mg (1.0 mmol) *p*-toluenesulfonyl chloride in 0.30 ml of dry pyridine was added to a cold (4°) solution of 250 mg (0.60 mmol) of the bicyclic oxime **9** in 25 ml of absolute CH_2Cl_2. After stirring for 15 h the mixture was diluted with H_2O (15 ml) and CH_2Cl_2 (15 ml). The solution was cautiously acidified with dilute HCl and the aqueous layer was extracted twice with CH_2Cl_2. The combined organic extracts were washed with H_2O, dried ($MgSO_4$), and concentrated. The residue (400 mg) was chromatographed on Si gel (30 g, petroleum ether-Et_2O, 99.4:0.6, to give 34.8 mg of **10** (13.8%) and 32.8 mg of **11** (13.0%).

Nitrile [**10**].—^{1}H nmr (80 MHz) δ 5.75 (1H, t, J=4 Hz, H-8), 5.3–4.9 (3H, m, H-14, H-18, H-22), 5.0 (1H, br s, H-1), 4.9 (1H, br s, H-1), 2.4–1.7 (20H, m), 1.62 (6H, s, H_3-24, H_3-25), 1.58 (9H, s, H_3-28, H_3-29, H_3-30), 1.02 (3H, s, H_3-26), 0.84 (3H, d, J=5 Hz, H_3-27); eims m/z $[M]^+$ 421 (0.7), 284 (3), 228 (2), 202 (3), 174 (4), 160 (11), 147 (19), 136 (9), 119 (11), 107 (13), 95 (17), 81 (42), 69 (100), 55 (21), 41 (57).

Nitrile [**11**].—^{1}H nmr (80 MHz) δ 6.22 (1H, br d, J=11 Hz, H-8), 5.25 (1H, br d, J=11 Hz, H-9), 5.14–4.9 (3H, m, H-14, H-18, H-22), 2.4–1.9 (18H, m), 1.8 (3H, s, H_3-1 or H_3-25), 1.74 (3H, s, H_3-1 or H_3-25), 1.62 (3H, s, H_3-30), 1.58 (9H, s, H_3-28, H_3-29, H_3-30), 1.02 (3H, s, H_3-26), 0.88 (3H, d, J=6 Hz, H_3-27); eims m/z $[M]^+$ 421 (0.5), 284 (1), 228 (2), 202 (3), 174 (4), 160 (9), 147 (14), 136 (8), 119 (10), 107 (13), 95 (18), 81 (40), 69 (100), 55 (21), 41 (55).

Monocyclic alcohol [**13**].—The nitrile **10** (16 mg; 0.038 mmol) was dissolved in 7 ml 20% ethanolic KOH, containing 2 drops of H_2O, and the solution was stirred for 1.5 h at 85–90°. After cooling to room temperature, the black reaction mixture was diluted with iced H_2O and acidified with HCl. After repeated extraction with petroleum ether/Et_2O, the combined organic layers were dried ($MgSO_4$) and evaporated to yield the acid **12** as a yellow oil, which was dissolved in 4 ml absolute Et_2O. Next, 30 mg (0.8 mmol) of $LiAlH_4$ were added, the solution was stirred at room temperature for 2 h, and then diluted with some Et_2O. Excessive $LiAlH_4$ was destroyed by dropwise addition of H_2O. The mixture was acidified with 2 N H_2SO_4, extracted with Et_2O, and the combined organic phases were washed with H_2O and dried over $MgSO_4$. Evaporation of the solvent followed by chromatography on Si gel (petroleum ether-Et_2O, 96.5:3.5 afforded the alcohol **13** (4.9 mg, 30.3%) as a colorless oil: eims m/z $[M]^+$ 426 (1.2), 311 (0.5), 285 (2), 233 (1), 208 (7), 189 (5), 161 (12), 147 (50), 133 (11), 119 (23), 107 (21), 95 (18), 81 (37), 69 (100), 55 (28), 41 (69).

Monocyclic alcohol [**14**].—Lithium granules (20 mg; 2.9 mmol) were dissolved in 2 ml of liquid NH_3 and two drops of EtOH were added. After dropwise addition of 16.6 mg (0.039 mmol) of the alcohol **13** in 1.5 ml absolute THF the solution was stirred for 4 h at −78°. The reaction mixture was quenched with 0.5 ml EtOH and allowed to warm to room temperature. The residue was diluted with Et_2O, washed twice with H_2O, dried ($MgSO_4$), and concentrated to give a crude product, which was chromatographed on Si gel (petroleum ether-Et_2O, 97:3) to give 1,10-dideoxyiridal **14** (7.4 mg, 44.6%) as a colorless oil. ^{1}H nmr (300 MHz) δ 5.1–5.01 (3H, m, H-14, H-18, H-22), 3.59 (2H, t, J=6.3 Hz, H_2-3), 2.54 (1H, m, H-6), 2.43 (2H, m), 2.1–1.9 (8H, m), 1.9–1.75 (2H, m), 1.66 (3H, s, H_3-24), 1.65 (3H, s, H_3-1 or H_3-25), 1.64 (3H, s, H_3-1 or H_3-25), 1.58 (6H, s, H_3-18, H_3-29), 1.57 (3H, s, H_3-30), 1.5–1.05 (6H, m), 0.88 (3H, s, H_3-26), 0.74 (3H, d, J=6.8 Hz, H_3-27); ^{13}C nmr (75 MHz) δ 134.9 (s, C-19), 134.2 (s, C-15), 131.6 (s, C-23), 131.2 (s, C-2 or C-7), 125.7 (d, C-14), 124.4 (d, C-18), 124.3 (d, C-22), 123.4 (s, C-7 or C-2), 63.7 (t, C-3), 44.3 (d, C-6), 39.7 (2C, t, C-16, C-20), 39.1 (s, C-11), 36.2 (d, C-10), 31.7 (t, C-12), 31.2 (t, C-4), 31.0 (t, C-9), 26.8 (t, C-21), 26.6 (t, C-17), 25.7 (q, C-24), 24.8 (t, C-8), 24.3 (q, C-26), 23.4 (t, C-5), 22.4 (t, C-13), 20.9 (q, C-1 or C-25), 20.2 (q, C-1 or C-25), 17.7 (q, C-30), 16.0 (q, C-29), 15.9 (q, C-28), 15.6 (q, C-27); eims m/z $[M]^+$ 428 (0.4), 291 (1.5), 210

(5.5), 167 (8), 149 (10), 136 (14), 123 (17), 109 (21), 95 (49), 81 (50), 69 (100), 55 (23), 41 (40).

Monocyclic aldehyde [**5a**].—CrO_3 (320 mg) was added in small portions to a mixture of 7.5 ml CH_2Cl_2 and 0.5 ml dry pyridine. After stirring for 15 min at room temperature, 0.3 ml of the solution were added to 6 mg (0.014 mmol) of the alcohol **14**, dissolved in 0.2 ml CH_2Cl_2. The mixture was stirred for 15 min at room temperature and a slurry of 300 mg Si gel in 5 ml petroleum ether-Et_2O (1:1) was added. The solution, obtained by filtration, contained the aldehyde **5a** as major product, which was characterized by gc-ms. Eims *m/z* $[M]^+$ 426 (0.4), 217 (0.4), 189 (3), 149 (5), 135 (16), 123 (10), 109 (13), 95 (26), 81 (36), 69 (100), 55 (20), 41 (42).

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