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ENT-KAURANE DITERPENOIDS FROM THE LIVERWORT *JUNGERMANNIA INFUSCA*

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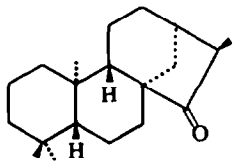
Key Word Index—*Jungermannia infusca*; Hepaticae; (16R)-*ent*-kauran-15-one; *ent*-kauren-15-one; *ent*-15α-hydroxykaurene; chemotaxonomy.

We have, recently, investigated the sesquiterpenoids of several liverworts and characterized enantiomeric forms of those from the higher plants [1]. Regarding the diterpenoids of the liverworts, there are few reports, i.e. of the isolation of *ent*-manool [2], five *ent*-kaurane-type diterpenoids [3, 4] and three *ent*-pimarane diterpenoids [5]. The present paper deals with the isolation and structural elucidation of three *ent*-kaurane diterpenoids from *Jungermannia infusca* (Mitt.) Steph. a leafy liverwort in the Jungermanniaceae. The neutral fraction of extract from the liverwort gave three diterpenoids (1–3) which were isolated along with a mixture of diterpene acetates.



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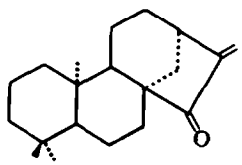
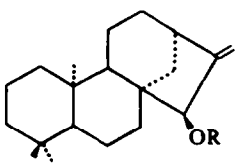


2

(*16R*)-ent-*Kauran-15-one* (1). The middle polar compound (R_f 0.51), $C_{20}H_{32}O$ (M^+ 288), mp 145–147°, $[\alpha]_D$ −76°, was characterized by spectral data as a saturated tetracyclic diterpenoid containing three tertiary methyls (ν 1390, 1375 cm^{-1}; δ 0.83, 0.90, 1.10, each 3H, *s*), a secondary methyl (δ 1.11, 3H, *d*, *J* = 6) and a partial structure of cyclopentanone (ν 1735 cm^{-1}). The compound was identified as (16R)-*ent*-kauran-15-one (1) by coincidence of the physical constants and spectral data [6].



3 R = H
4 R = Ac

ent-*Kauren-15-one* (2). The least polar diterpenoid (R_f 0.58), $C_{20}H_{30}O$ (M^+ 286), mp 99–100°, $[\alpha]_D$ −220°, was also assumed to be a kaurane type ketone containing three tertiary methyls (ν 1377, 1370 cm^{-1}; δ 0.83, 0.90, 1.10, each 3H, *s*) and a partial structure of a cyclopentanone conjugated with an exomethylene (ν 1725, 1645, 935 cm^{-1}; δ 5.25, 5.95, each 1H, *br.s*; λ_{max}^{EtOH} 233 nm, ε 8800). On hydrogenation over Adams catalyst in ethyl acetate it gave 1. Thus, the compound was identified as *ent*-kauren-15-one (2).

ent-*15α-Hydroxykaurene* (3). The most polar compound (R_f 0.42), $C_{20}H_{32}O$ (M^+ 288), mp 99–100°, $[\alpha]_D$ −70°, was isolated as a major component. The spectral data exhibited the presence of a secondary hydroxyl group (ν 3600, 3450 cm^{-1}; δ 3.97, 1H, *br*), three tertiary methyls (ν 1393, 1375 cm^{-1}; δ 0.82, 0.87, 1.03, each 3H, *s*) and an exomethylene (ν 1665, 895 cm^{-1}; δ 4.87, 5.00, each 1H, *br.s*). In addition, the compound was treated with Ac_2O in pyridine to give an acetate (4), $C_{22}H_{34}O_2$ (M^+-330); ν 1740, 1665, 890 cm^{-1}; δ 0.82, 0.87, 1.05, 2.12 (each 3H, *s*), 4.86 (1H, *br*), 4.86, 5.07 (each 1H, *br.s*), and it was oxidized with Jones reagent to give the α,β-unsaturated ketone 2. Thus, it was identified as kauren-15-ol. In order to determine the configuration of the hydroxyl group located on C-15 in the *ent*-kaurene skeleton, the alcohol (3) was treated with HCl to afford 1 through a ready garryfoline–cuauchichine rearrangement [7, 8]. Thus the stereostructure of the alcohol is *ent*-15α-hydroxykaurene (3).

Although the three *ent*-kaurane diterpenoids have been reported as reaction products, this is their first isolation as natural products. Their biosynthetic simplicity [9] fits in with their occurrence in a very ancient group of plants.

EXPERIMENTAL

Mp's are uncorrected. IR and PMR spectra were determined in CCl_4 or $CHCl_3$, and the mass spectra on a single focusing instrument under the following conditions: 70 eV ionization chamber voltage, 80 μA total emission, 1800 V accelerating voltage and 200° ionization chamber temperature. The optical rotations were measured with an automatic polarimeter in $CHCl_3$.

Material and isolation. The liverwort (1.7 kg) collected at the suburbs of Owase City in Mie Prefecture was dried and digested with MeOH to obtain a crude extract (37.9 g). A neutral part (23.7 g) of the extract was subjected to Si gel-column chromatography followed by preparative TLC with Si gel in hexane–C_6H_6 (1:2) to give 3 crystalline compounds, 1 (R_f 0.51), 2 (R_f 0.58) and 3 (R_f 0.42), which were further purified by recrystallization from EtOH.

Catalytic hydrogenation of 2 *to* 1. Unsaturated ketone (2, 15 mg) was hydrogenated over PtO_2 (3 mg) in EtOAc (7 ml) at room temp., and the reaction mixture, on working up, gave a crystalline product from EtOH: $C_{20}H_{32}O$ (M^+ 288), mp 148–149°, $[\alpha]_D$ −77°.

Oxidation of 3 *to* 2. The alcohol (3, 20 mg) in Me_2CO (3 ml) was treated with an excess of Jones reagent at 0° for 5 min. The product extracted with Et_2O was submitted to preparative TLC to give the α,β-unsaturated ketone (2, 15 mg) which was crystallized from EtOH as needles: $C_{20}H_{30}O$ (M^+ 286), mp 99–100°, $[\alpha]_D$ −146°.

Acetylation of 3 *to* 4. The diterpenoid (3, 10 mg) was treated with Ac_2O (0.5 ml) in dried pyridine (0.5 ml) at room temp for 15 hr. The product 4 (7 mg) $C_{22}H_{34}O_2$ (M^+ 330) had mp 115–116°, $[\alpha]_D$ −100°.

Rearrangement of 3 *to* 1. The unsaturated alcohol (3, 20 mg) in MeOH (4 ml) and ether (2 ml) was treated with conc NCl (0.8 ml) at room temp. for 3 hr. The reaction mixture was taken up into Et_2O, and the ethereal solution was washed, dried and concd to give a major product (1, 15 mg) which was submitted to preparative TLC and recrystallization: $C_{20}H_{32}O$ (M^+ 288), mp 148–149°, $[\alpha]_D$ −78°.

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IN VITRO CYCLIZATION OF SQUALENE 2,3-EPOXIDE TO α-AMYRIN BY MICROSOMES FROM BRAMBLE CELL SUSPENSION CULTURES

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Key Word Index—*Rubus fruticosus*; Rosaceae; squalene 2,3-epoxide-α-amyrin cyclase.

Abstract—Squalene 2,3-epoxide incubated with microsomes from bramble cell suspension cultures is shown to be converted into α-amyrin.

INTRODUCTION

In 1955, Ruzicka and his coworkers proposed the biosynthesis of α- and β-amyrin, along with other triterpenes and steroids from squalene [1]. The *in vitro* incorporation of mevalonate and squalene 2,3-epoxide into β-amyrin has been demonstrated [2–5]. In the case of α- amyrin, *in vivo* experiments have shown incorporation of CO_2, acetate and mevalonate into α-amyrin or its derivatives [6–8]. Recently incorporation of mevalonate into α- and β-amyrin by chopped preparations of plant parts has been shown [9]. *In vitro* biosynthesis of α-amyrin has not been reported; however, Corey and Dean imply that less than 5% of the radioactivity found in impure β-amyrin from *in vitro* incorporation of squalene-epoxide might be due to α-amyrin but offer no proof [5, 10]. In many cases, α-amyrin seems to constitute a minor component of the pentacyclic triterpene mixture and this, added to the difficulty to separate the two amyrins, rendered very difficult the study of *in vitro* biosynthesis of α-amyrin. As we recently showed that α-amyrin constituted more than 70% of the pentacyclic triterpene fraction in bramble cells [11], we decided therefore to study the *in vitro* biosynthesis of this compound. We wish to report here an unambiguous incorporation of squalene epoxide into α-amyrin by microsomes from bramble cells suspension cultures.

RESULTS AND DISCUSSION

The 4,4-dimethyl steryl acetate fraction from bramble cell suspension cultures has been resolved by argentation chromatography into 24-methylene cycloartenyl acetate (R_f 0.20), cycloartenyl acetate (R_f 0.45) and pentacyclic triterpene acetates (R_f 0.80). GC–MS analysis showed that this latter fraction contained β-amyrin