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STRUCTURE OF INFLEXINOL, A NEW CYTOTOXIC DITERPENE FROM *RABDOSIA INFLEXA*

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Key Word Index—*Rabdosia inflexa*; Labiatae; cytoxic diterpenoids; inflexinol; ent-kaurenoid; ent-kaur-16-en-15-one-1α,3α,6β,11β-tetraol 1,3-diacetate.

Abstract—From the leaves of *Rabdosia inflexa* a new cytotoxic diterpenoid, inflexinol was isolated, together with the known inflexin, and the structure was established as ent-kaur-16-en-15-one-1α,3α,6β,11β-tetraol 1,3-diacetate.

INTRODUCTION

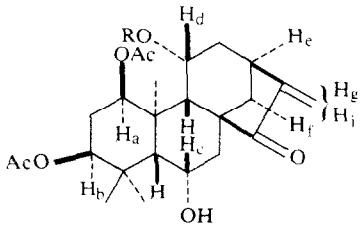
From the various plants, belonging to the genus *Rabdosia* [1] (Labiatae), many diterpenoids of the ent-kaurene, B-seco-ent-kaurene and B/C-seco-ent-kaurene types were isolated and their structures were determined [2, 3]. Recently, antitumor and antibacterial activities of these diterpenoids were examined [4, 5]. During the investigations of biologically active substances, we have examined the constituents of the leaves of *Rabdosia inflexa* and isolated a new minor diterpenoid, inflexinol (**1**) together with the known inflexin (**2**) [6]. Inflexinol (**1**) showed a cytotoxic activity against cultured rat mammary cancer FM 3A/B cells. We report here the structure elucidation of inflexinol (**1**).

RESULTS AND DISCUSSION

Inflexinol (**1**) was obtained from the methanolic extract of the leaves of *R. inflexa* as an amorphous powder, $[\alpha]_D^{28} -43.0°$ (MeOH; *c* 0.22) and had a molecular formula, $C_{24}H_{34}O_7$, from high resolution mass spectroscopy. Inflexinol (**1**) showed an absorption maximum at 237 nm (ε 6124) in the UV, absorptions at 1720 and 1645 cm^{-1} in the IR and signals at δ 5.29 and 5.90 (each 1 H, each *br s*) in the ^{1}HNMR spectrum. These spectral data suggested that **1** had a five membered ketone conjugated with an α-methylene group as a partial structure. Besides the signals of three tertiary methyl groups at δ 0.91, 1.28 and 1.49 and the signals of two acetyl groups at 1.90 and 2.07, the ^{1}H NMR spectrum showed the signals due to two protons attached to carbons having an acetoxy group at 4.71 (*t*, *J* = 3 Hz, H_b) and 5.90 (*t*, *J* = 3 Hz, H_a) and the signals assigned to protons attached to a hydroxy group bearing carbons at 3.69 (*dd*, *J* = 10 and 6 Hz, H_d) and 4.43 (*dd*, *J* = 5 and 3 Hz, H_c). From these facts, it was presumed that inflexinol (**1**) had a 15-oxo-ent-kaurene as a basic skeleton which is common in the diterpenoids of *Rabdosia* plants. In fact, dihydroinflexinol (**3**) showed a negative Cotton effect in its ORD spectrum. Comparing the ^{1}H NMR spectrum of **1** with that of **2**, the proton signals due to H_a, H_b and H_d in **1** were observed at the corresponding position in **2**, but the new proton signal due to H_c also appeared in the spectrum of **1**. On the other hand, the signals at δ 2.71 (*s*, 5-H) and 3.12 (*d*, *J* = 12 Hz, 7α-H) in the spectrum of **2** were missing in that of **1**. These results suggest that the carbonyl group at C-6 in **2** had been reduced to an alcoholic group in **1**. We have further examined the probability of this presumption by INDOR (internuclear double resonance) [7] experiments.

On monitoring H_g or H_i, the INDOR signals were observed at H_e and H_i, or H_e and H_g. These results support the structure of the D-ring. When H_b was monitored, an INDOR signal due to coupling was observed in the same methylene region (δ 1.60–2.40) as on monitoring H_a and the signals due to NOE's were also observed on both the methyl groups at C-4 (δ0.91 and 1.28). These results suggested that **1** had β-axial acetoxy groups at C-1 and C-3 in the A-ring as in the case of **2**. On monitoring H_c, INDOR signals due to coupling were observed around δ 1.29–1.42 and 2.00–2.29 and the signals due to NOE's were observed for methyl groups at C-4. Considering the coupling constant of H_c, this result suggested that H_c was a β-equatorial proton at C-6, namely, an α-axial hydroxy group was located at that position. Finally, a signal arising from NOE was observed on H_f (δ 2.91, *d*, *J* = 13 Hz, 14α-H) when monitored from a methyl group at C-10 (δ 1.49). From the above-mentioned facts, the structure of inflexinol must be ent-kaur-16-en-15-one-1α,3α,6β,11β-tetraol 1,3-diacetate (**1**).

In order to verify the presumed structure, inflexinol (**1**) was chemically correlated with inflexin (**2**). Acetylation of **1** with Ac_2O–pyridine gave 11-monoacetate (**4**), mp 247–248° (δ 4.84, *dd*, *J* = 12 and 6 Hz, 11β-H). The fact that a hydroxy group at C-6 was not acetylated agrees with the presumption that the configuration of the hydroxy group is α-axial.



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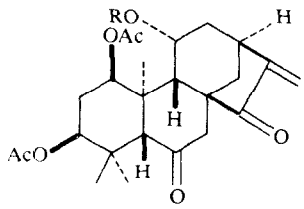

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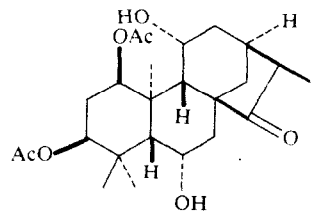

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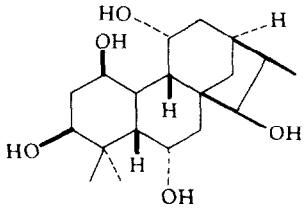

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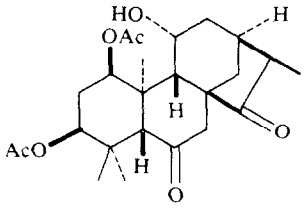

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Jones oxidation of **4** gave inflexin acetate (**5**). When dihydroinflexinol (**3**) was subjected to $LiAlH_4$ reduction, a pentahydroxy-ent-kaurane (**6**), mp 227–229°, was obtained. From the consideration of the stereomodel, it is expected that 6α-axial hydroxy group should be formed by $LiAlH_4$ reduction of dihydroinflexin (**7**) as in the case of 6-ketosteroids [8]. In fact, the product obtained from **7** was identical with **6**. Accordingly, the structure of inflexinol was established as **1**.

EXPERIMENTAL

General procedures. All mps are uncorr. ^{1}H NMR: 100 or 90 MHz. All the ^{1}H NMR spectra were taken for $CDCl_3$ solns unless otherwise noted. Chemical shifts are given in δ with TMS as int. standard. MS: 70 eV unless otherwise noted.

Plant material. Plants were collected in the suburbs of Tokushima City (Tokushima Pref., Japan) in Oct. 1976 and identified as *Rabdosia inflexa* (Thunb.) Hara by Mr. G. Murata of Faculty of Sciences, Kyoto University. A voucher specimen (T. Fujita No. 12) was deposited in the Herbarium of the Institute of Botany, Kyoto University (KYO), Kitashirakawaoiwake-cho, Sakyo-ku, Kyoto 606, Japan.

Isolation procedures. Methanolic extracts obtained from the dried leaves of *Rabdosia inflexa* (6.2 kg) were combined and concd *in vacuo*. The residue was dissolved in 90% MeOH (3 l.) and then the soln was washed ×3 with *n*-hexane (total 4.5 l.). The 90% MeOH layer was concd *in vacuo*. The residue was suspended in H_2O (1.6 l.) and extracted with EtOAc (total 7.5 l.). After washing with H_2O, the EtOAc extract was dried and evaporated *in vacuo* to give a residue (180.6 g). This residue was chromatographed on a Si gel (3 kg) column with $CHCl_3$–MeOH with increasing MeOH content. The fraction eluted with $CHCl_3$–MeOH (97:3) gave a residue (49.11 g), which contained inflexinol (**1**) and inflexin (**2**). The residue was further separated and purified by repeated Si gel chromatography using Et_2O as eluent to give inflexinol (**1**) (0.435 g) and inflexin (**2**) (7.72 g).

Inflexinol (**1**); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600, 3550–3350, 1720, 1645, 1260; ^{1}HNMR: δ 0.91, 1.28, 1.49 (3 × *s*, 3 × tert. Me), 1.90, 2.07 (2 × *s*, 2 × OAc), 2.91 (*d*, *J* = 13 Hz, 14α-H), 3.11 (*m*, 13-H), 3.69 (*dd*, *J* = 12 and 5 Hz, 11-H), 4.43 (*dd*, *J* = 5 and 3 Hz, 6-H), 4.71 (*t*, *J* = 3 Hz, 3-H), 5.29 (*br s*, 17-H_1), 5.90 (*t*, *J* = 3 Hz, 1-H), 5.90 (*br s*, 17-H_1); MS *m/z*: M^+ 434.230 (calc. for $C_{24}H_{34}O_7$ 434.230). Inflexinol (**1**) at 10 μg/ml showed inhibitory activity (89.5%) on the growth of cultured rat mammary cancer FM 3A/B cells.

Inflexin (**2**); recrystallized from a mixture of Et_2O and *n*-hexane, mp 202–204°, $[\alpha]_D^{29}$ −57.1° (MeOH; *c* 1.0); UV λ_{max}^{MeOH} nm (ε): 238 (7708); IR ν_{max}^{KBr} cm^{-1}: 3375, 1730, 1705, 1650, 1260; ^{1}H NMR: δ 0.86, 1.16, 1.32 (3 × *s*, 3 × tert. Me), 1.96, 2.12 (2 × *s*, 2 × OAc), 2.32 (*d*, *J* = 12 Hz, 14α-H), 2.71 (*s*, 5-H), 3.10 (*m*, 13-*H*), 3.12 (*d*, *J* = 12 Hz, 7α-H), 3.94 (*dd*, *J* = 12 and 6 Hz, 11-H), 4.56 (*t*, *J* = 3 Hz, 3-H), 5.34 (*br s*, 17-H_1), 5.95 (*t*, *J* = 3 Hz, 1-H), 5.95 (*br s*, 17-H_1); MS *m/z*: 432.217 $[M]^+$ (calc. for $C_{24}H_{32}O_7$ 432.215). The physical properties of this substance agree with those of inflexin (**2**) [6].

Catalytic hydrogenation of inflexinol (**1**). Inflexinol (**1**) (85 mg) was dissolved in MeOH (8 ml) and hydrogenated over PtO_2 (4 mg) for 30 min. The catalyst was filtered off and the solvent removed *in vacuo* to give dihydroinflexinol (**3**) (74 mg). ORD λ_{max}^{MeOH} nm ([φ]): 322 (−1831), 288 (+1716); IR ν_{max}^{KBr} cm^{-1}: 3400, 2900, 1720, 1370, 1250; ^{1}H NMR: δ 0.91, 1.27, 1.48 (3 × *s*, 3 × tert. Me), 1.30 (*d*, *J* = 7 Hz, 16-Me), 1.99, 2.07 (2 × *s*, 2 × OAc), 2.88 (*d*, *J* = 12 Hz, 14α-H), 3.64 (*dd*, *J* = 12 and 6 Hz, 11-H), 4.36 (*m*, 6-H), 4.67 (*t*, *J* = 3 Hz, 3-H), 5.76 (*m*, 1-H); MS (22 eV) *m/z*: 418.237 $[M-H_2O]^+$ (calc. for $C_{24}H_{34}O_6$ 418.235).

Acetylation of inflexinol (**1**). Inflexinol (**1**) (50 mg) was acetylated with Ac_2O–pyridine at room temp. for 48 hr and the product purified on a Si gel column with $CHCl_3$ as eluent and recrystallized from a mixture of Et_2O and *n*-hexane to give inflexinol 11-acetate (**4**) (18 mg) as colourless plates, mp 247–248°. IR ν_{max}^{KBr} cm^{-1}: 3450, 2900, 1730, 1645, 1250; ^{1}H

NMR: δ 0.94, 1.30, 1.60 (3 × *s*, 3 × tert. Me), 1.78, 1.99, 2.09 (3 × *s*, 3 × OAc), 2.81 (*d*, *J* = 12 Hz, 14α-H), 3.06 (*m*, 13-H), 4.44 (*m*, 6-H), 4.64 (*t*, *J* = 3 Hz, 3-H), 4.85 (*dd*, *J* = 10 and 6 Hz, 11-H), 5.19 (*br s*, 17-H_1), 5.60 (*m*, 1-H), 5.84 (*br s*, 17-H_1) (Found C, 65.23; H, 7.88. $C_{26}H_{36}O_8$ requires: C, 65.53; H, 7.61%).

Acetylation of inflexin (**2**). Inflexin (**2**) (300 mg) was acetylated with Ac_2O–pyridine at room temp. for 84 hr to give inflexin acetate (**5**) (296 mg). $[\alpha]_D^{28}$ − 28.9° (MeOH; *c* 0.27); IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1740, 1720, 1645, 1370, 1245; ^1H NMR: δ 0.90, 1.25, 1.34 (3 × *s*, 3 × tert. Me), 1.84, 2.02, 2.13 (3 × *s*, 3 × OAc), 2.84 (*s*, 5-H), 3.06 (*m*, 13-H), 3.22 (*d*, *J* = 12 Hz, 7α-H), 4.57 (*t*, *J* = 3 Hz, 3-H), 5.08 (*dd*, *J* = 12 and 6 Hz, 11-H), 5.31 (*br s*, 17-H_1), 5.60 (*m*, 1-H), 5.95 (*br s*, 17-H_1); MS *m/z*: 474.224 $[M]^+$ (calc. for $C_{26}H_{34}O_8$ 474.225).

Jones oxidation of inflexinol 11-acetate (**4**). A soln of **4** (18 mg) in Me_2CO (2 ml) was stirred with Jones reagent (5 drops) under ice cooling for 30 min. The reaction mixture was diluted with excess H_2O and extracted with $CHCl_3$. The $CHCl_3$ extract was dried and evaporated *in vacuo*. The residue (11 mg) was purified by Si gel TLC (CH_2Cl_2–Me_2CO, 9:1) to give an oxidation product (3.8 mg). $[\alpha]_D^{28}$ − 16.9° (MeOH; *c* 0.21); MS *m/z*: 414.203 $[M-AcOH]^+$ (calc. for $C_{24}H_{30}O_6$ 414.204). This substance was identified with an authentic sample of inflexin acetate (**5**) by comparison of IR and ^1H NMR spectra.

Reduction of dihydroinflexinol (**3**) *with* $LiAlH_4$. To a soln of dihydroinflexinol (**3**) (32 mg) in dry THF (10 ml), $LiAlH_4$ (32 mg) was added and the reaction mixture was stirred at room temp. for 24 hr. After successive addition of excess EtOAc, excess H_2O and dil. H_2SO_4 (until the resulting ppt. was dissolved), the resulting EtOAc phase was separated. The EtOAc extract was washed with H_2O, dried and evaporated *in vacuo* to give a residue (18 mg), which was purified by Si gel TLC ($CHCl_3$–MeOH, 9:1) and recrystallized from MeOH to give a pentahydroxy-ent-kaurane (**6**) (7.6 mg), mp 227–229°. IR ν_{max}^{KBr} cm^{-1}: 3600–3000, 2860, 1060, 1030; ^1H NMR (d_5-pyridine): δ 1.36, 1.64, 2.04 (3 × *s*, 3 × tert. Me), 1.46 (*d*, *J* = 8 Hz, 16-Me), 3.76 (1 H, *m*), 3.90 (1 H, *d*, *J* = 12 Hz), 4.72 (2 H, *m*), 5.08 (1 H, *m*); MS *m/z*: 336.234 $[M-H_2O]^+$ (calc. for $C_{20}H_{32}O_4$ 336.230).

Catalytic hydrogenation of inflexin (**2**). Inflexin (**2**) (100 mg) was dissolved in MeOH (5 ml) and hydrogenated over PtO_2 (5 mg) for 30 min. The catalyst was filtered off and solvent was removed *in vacuo* to give dihydroinflexin (**7**) (100 mg). IR ν_{max}^{KBr} cm^{-1}: 3250, 2850, 1730, 1715, 1365, 1230; ^1H NMR: δ 0.86, 1.12, 1.28 (3 × *s*, 3 × tert. Me), 1.29 (*d*, *J* = 6 Hz, 16-Me), 2.01, 2.04 (2 × *s*, 2 × OAc), 2.34 (*d*, *J* = 12 Hz, 14α-H), 2.67 (*s*, 5-H), 3.02 (*d*, *J* = 12 Hz, 7α-H), 3.98 (*dd*, *J* = 12 and 6 Hz, 11-H), 4.58 (*t*, *J* = 3 Hz, 3-H), 5.80 (*t*, *J* = 3 Hz, 1-H); MS *m/z*: 434.230 $[M]^+$ (calc. for $C_{24}H_{34}O_7$ 434.230).

Reduction of dihydroinflexin (**7**) *with* $LiAlH_4$. To a soln of dihydroinflexin (**7**) (50 mg) in dry THF (5 ml), $LiAlH_4$ (50 mg) was added and the reaction mixture was stirred at room temp. for 4 hr. The reaction mixture was worked up as before and the product (20 mg) was recrystallized from MeOH to give pentahydroxy-ent-kaurene (**6**) (8 mg), mp 226–229°; MS *m/z*: 336.233 $[M-H_2O]^+$ (calc. for $C_{20}H_{32}O_4$ 336.230). This substance was identified with **6**, derived from dihydroinflexinol (**3**), by mmp and comparisons of IR and ^1H NMR spectra.

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