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THE STRUCTURE OF INFLEXIN

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The structure of inflexin isolated from the leaves of Isodon inflexus has been determined as 1 by spectroscopic and chemical methods. Inflexin exhibits cytotoxicity, insect antifeedant and other bioactive properties.

Chemical examinations of Isodon species have produced a number of biological active ent-kaurenoids.[1] In this communication we report the structure of inflexin isolated from the dry leaves of Isodon inflexus (Labiatae) in 0.0003% yield. Inflexin possesses in vitro cytotoxicity (KB),[2] inhibitory activity on the respiratory reactions of mitochondria from rat liver,[3] and specific toxicity to the Lepidoptera larvae.[4]

We propose ent-kaurene structure 1 for inflexin, which has the following physical properties, $C_{24}H_{32}O_7$ (CI/MS in isobutane and elemental analysis), m.p. 203-205°C, $[\alpha]_D^{19}$ -47° (c=1.0, EtOH), uv (EtOH) 238 nm (ε 8020), ir ($CHCl_3$) 3360 (hydroxyl), 1720 and 1260 (acetate), and 1690 and 1640 cm^{-1} (5-membered ring ketone conj. with exocyclic methylene). The cmr data of inflexin, as summarized in 1, showed the presence of three methyls, two acetoxyls, four methylenes, six methines, three tetra-substituted carbons, two olefinic carbons, and four carbonyl carbons.[5]

The above data and two broad singlets at 5.94 and 5.34 ppm in the pmr spectrum (100 MHz) suggest that inflexin has 15-oxo-16-kaurene skeleton which is typical of Isodon diterpenes.[6] Spindecoupling of 17-H's in 1 established the location of the 13-H multiplet at 3.10 ppm, which was also coupled to the two geminal protons at 2.30 (14α-H, dd, 12 and 1 Hz) and 1.54 ppm (14β-H, dd, 12 and 5 Hz). The 7α- and 7β-H's

appeared at 3.10 and 1.80 ppm, respectively , with a J_{gem} of 12 Hz, which indicated that they were adjacent to a carbonyl group. The appearance of the 7α-H signal in the lower field is accounted for by the deshielding effect of the 6-one. Irradiation of the 7α-H signal sharpened the 5β-H peak (br s at 2.68 ppm).

The uncommonly low chemical shift of 1α-H (5.95 ppm, t, 3 Hz) can be rationalized by the deshielding effect of the 11α-hydroxyl group. The 11α-hydroxyl configuration is corroborated by the presence of J values for the 11β-H peak (10 and 6 Hz, and no coupling between 11β-H and 12β-H) at 3.94 ppm.[7] This stereochemical relationship was further supported as follows. Dihydroinflexin 2[8] prepared by catalytic hydrogenation of 1, upon oxidation with Jones' reagent yielded the 6,11,15-trione 3. The pmr of 3 exhibited the 1α-H peak at the expected chemical shift (4.93 ppm), thus clearly pointing to the presence of an 11α-hydroxyl group in 1. The 10-methyl resonance exhibited a paramagnetic shift from 0.85 to 1.46 ppm. The pmr data of 3α-H (4.56 ppm, t, 3 Hz) clearly indicated that the remaining acetoxyl group is linked to C-3 and is β. Accordingly, the ring A presumably adopts a twist boat conformation to relieve two 1,3-diaxial interactions in the chair conformation.

The gross structure of ring A/B/C was established by observation of 18% NOE on the 10-methyl signal upon irradiation of the 14α-H signal. Finally, the elucidation of the absolute configuration rested on the cd curve ($\Delta\varepsilon_{300}$ -0.65 in MeOH) of dihydro-6-deoxoinflexin 4[9] on comparison of the sign with it of other authentic samples of ent-kaurenoid-15-one.[10,11]

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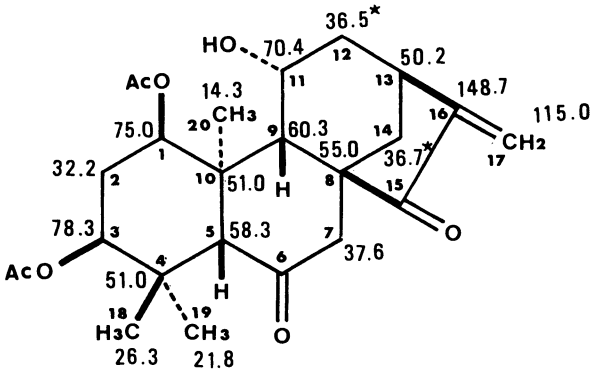
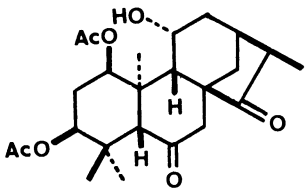


1 Cmr of inflexin in $CDCl_3$; C=O at 209.9, 205.5, 171.4 and 170.1 ppm; * assignments interchangeable



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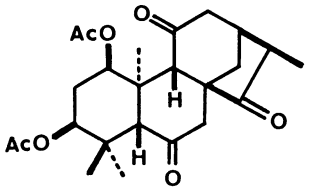

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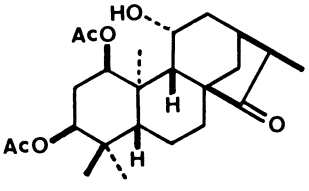

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REFERENCES AND FOOTNOTES

1. I. Kubo, M. Taniguchi, Y. Satomura, and T. Kubota, Agr. Biol. Chem., 38, 1261 (1974)

2. The cytotoxicity (KB) effect (LD_{50}) was 5.4 μg/ml. We thank Professor F. J. Schmitz, Department of Chemistry, University of Oklahoma, for taking care of bioassay.

3. N. Abo-Khatwa and I. Kubo, tobe published.

4. It was suspected that inflexin had antiecdysone activity against army worms. However, injection tests carried out Dr. T. Ohtaki showed that this was not the case.

5. A JEOL PS-100 instrument was used. The results are based on a combination of PND, off-resonance decoupling and PRFT/off-resonance decoupling techniques. See, I. Miura, K. Nakanishi, and D. L. Elder, J. Amer. Chem. Soc., 97, 1975 (1975)

6. I. Kubo, T. Kamikawa, and T. Kubota, Tetrahedron, 30, 615 (1974)

7. Addition of the pmr shift reagent $Eu(dpm)_3$ caused a clear downfield shift of 9β-H (d, 6 Hz). The $J_{11\beta,12\beta}$ is hence zero.

8. Since the reagent is known to attack from the least hidered side, the newly formed 16-methyl of 2 is assigned the β (R)-configuration. The double irradiation experiments involving 16-H and 17-H's of 2 which led to the determination of $J_{13,16}$ as 7 Hz also supports the above conclusion. Satisfactory spectra and/or elemental analyses were obtained for all new compounds here reported.

9. Prepared by Raney-Ni desulfurization of dihydro-6-dithioketalinflexin.

10. W. Herz and R. P. Sharma, J. Org. Chem., 41, 1021 (1976)

11. I. Kubo, I. Miura, T. Kamikawa, T. Isobe, and T. Kubota, to be published.

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