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STRUCTURE OF CYCLOKESSYL ACETATE, A SESQUITERPENOID OF VALERIANA FAURIEI 'HOKKAI-KISSO' ROOTS[1]

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Abstract — A new sesquiterpenoid, cyclokessyl acetate, was isolated from the roots of Valeriana fauriei 'hokkai-kisso' (Valerianaceae), and its structure was determined by chemical and spectroscopic evidence, especially by means of two-dimensional NMR correlations, to have a novel carbon skeleton.

The crude drug "kisso-kon", prepared from the rhizomes and roots of Valeriana plants (Valerianaceae), has been used for sedative and antispasmodic purposes. We have hitherto performed analysis of the essential oil of this group of plants and isolated a number of new sesquiterpenoids, which have the guaiane and valerane skeletons.[2]

In continuation of our chemical work on Valeriana plants, a novel sesquiterpenoid has been isolated by conventional alumina and silica gel chromatographic methods from the essential oil of Valeriana fauriei 'hokkai-kisso' and given the trivial name cyclokessyl acetate. We describe here the elucidation of the stereostructure of cyclokessyl acetate.

Cyclokessyl acetate, mp 84-85.5°, $[\alpha]_D$ -46.4° (c 0.40, $CHCl_3$) was established to have the molecular formula $C_{17}H_{26}O_3$ from the molecular ion peak at m/z 278 in its EI-mass spectrum and the analysis of its ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) (CH_3- x 5, $-CH_2$- x 2, >CH- x 6, >C-O x 2, >CH-O x 1 and >C=O x 1) (Table I). The absence of olefinic carbon signals in the ^{13}C NMR spectrum, combined with the degree of unsaturation of the molecule, required cyclokessyl acetate to have a tetracyclic skeleton. The IR spectrum (KBr) of cyclokessyl acetate disclosed strong absorption bands at 1733 and 1227 cm^{-1} associated with an ester group, which, coupled with a three-hydrogen singlet at δ 1.98 in the ^1H NMR spectrum ($CDCl_3$, 500 MHz) and a mass fragment peak at m/z 218 (M^+-AcOH), indicated the presence of an acetoxyl group. From the absence of an IR absorption band for a hydroxyl group and the presence of two ^{13}C NMR signals (δ 72.2 (d x 1C) and 72.2 (s x 2C)) assignable to carbons attached to oxygen atoms, the remaining oxygen atom present in the molecule must constitute an ether linkage.

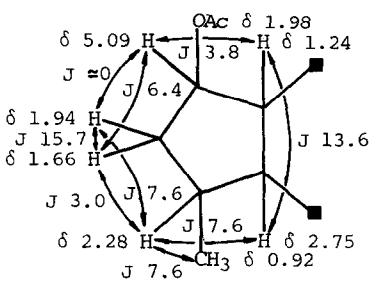
In the ^1H NMR spectrum, a three-hydrogen doublet at δ 0.92 (J 7.6 Hz) and three-hydrogen singlets at δ 1.13, 1.33 and 1.34 revealed the presence of one secondary methyl and three tertiary methyls, and the chemical shifts of the latter three signals suggested that they were on carbons bearing oxygen functions. A one-hydrogen triplet (J 8.5 Hz) appeared at δ 0.90, the resonance position of which was shifted slightly upfield compared with the range of typical methylene or methine hydrogens. This ^1H NMR signal, together with the observation of three shielded methine carbon signals at δ 11.2 (d), 13.4 (d) and 22.4 (d) in the ^{13}C NMR spectrum, indicated that cyclokessyl acetate possesses a cyclopropane ring. Further, the presence of the cyclopropane moiety was confirmed by the characteristically large J_{CH} values for each of these carbon signals (165, 160 and 157 Hz, respectively).

Examination of the ^1H shift correlation two-dimensional NMR spectrum (COSY spectrum),[3]

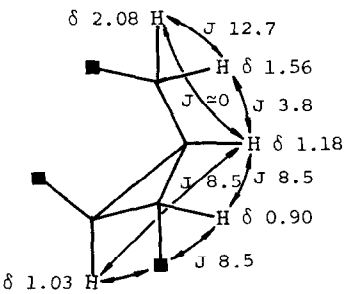
Table I. ^{13}C NMR data of cyclokessyl acetate (δ)

C-1	52.6 d	C-11	72.2 s
C-2	72.2 d	C-12	33.5 q
C-3	42.3 t	C-13	29.5 q
C-4	30.7 d	C-14	16.6 q
C-5	36.5 d	C-15	26.8 q
C-6	13.4 d	OAc	21.3 q
C-7	22.4 d		170.6 s
C-8	11.2 d		
C-9	34.7 t		
C-10	72.2 s		

with the aid of decoupling experiments, indicated the presence of the partial structure A. On alkali hydrolysis with 1 N potassium hydroxide in ethanol-water, cyclokessyl acetate yielded the alcohol (**2**) (IR (CCl_4): ν 3400 cm^{-1} (OH); ^1H NMR ($CDCl_3$, 60 MHz): δ 3.97 (1H triplet)), which was converted to the ketone (**3**) (MS m/z: 234 (M^+)) on chromic acid oxidation. The IR spectrum (KBr) of the ketone (**3**) showed an absorption band at 1730 cm^{-1} due to carbonyl group in a five-membered ring, supporting the presence of the partial structure A. Further, it was found that cyclokessyl acetate had the partial structure B through ^1H NMR double resonance experiments, in which the configurations of the hydrogens on the cyclopropane ring were deduced to be all cis based on the values for the vicinal coupling constants (J 8.5 Hz).[4]



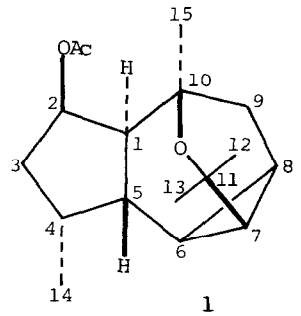
A



B

The remaining problem to be solved on the gross structure of cyclokessyl acetate was the connection of the partial structures A and B, two quaternary carbons forming the ether linkage, and three methyls on quaternary carbons. After completion of the assignments of the ^{13}C NMR signals through acquisition of a ^1H-^{13}C shift correlation two-dimensional NMR spectrum (H-C COSY spectrum) (Fig. 1),[5] the ^{13}C shift correlation two-dimensional NMR spectrum (2D INADEQUATE spectrum) (Fig. 2)[6] was analyzed. In this way, the connection between the C-5 methine carbon and C-6 methine carbon was unambiguously clarified. Further it was found that the C-1 and C-9 carbons and one tertiary methyl were bonded to the C-10 quaternary carbon, and that the C-7 carbon and two tertiary methyls were connected to the other quaternary carbon (C-11). These spectral data, along with the fact that α-kessyl alcohol and kessyl glycol were previously isolated from the same plant source,[7] allowed the molecular framework of cyclokessyl acetate to be established as shown in formula **1** (without stereochemistry).

The relative and absolute stereochemistry of cyclokessyl acetate was then examined. In the NOE difference spectrum of cyclokessyl acetate, significant NOE's were observed for the H-2 and H-9α signals by saturation of the H-1 signal, indicating a spacial proximity between H-2 and H-1, and H-9α and H-1. Detection of NOE's between the H-4 and H-5 signals, and the H-5 and H-13 signals demonstrated that H-5 is close to H-4 and H-13. Thus, the relative stereochemistry of the ring junction was deduced to be trans, and the relationships between H-1 and H-2, and H-4 and H-5 were both cis. The CD spectrum of the ketone (**3**), which showed a positive Cotton effect associated with the carbonyl function ($[\theta]_{299}$ +4300), allowed the



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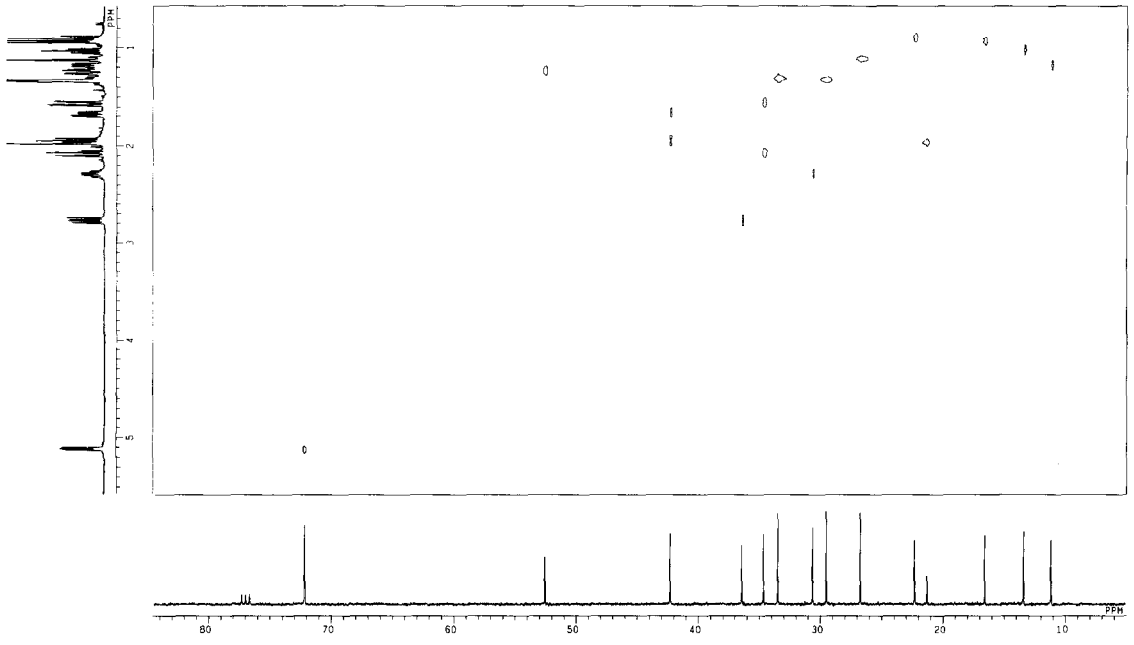

Fig. 1. ^{1}H-^{13}C shift correlation two-dimensional NMR spectrum of cyclokessyl acetate.

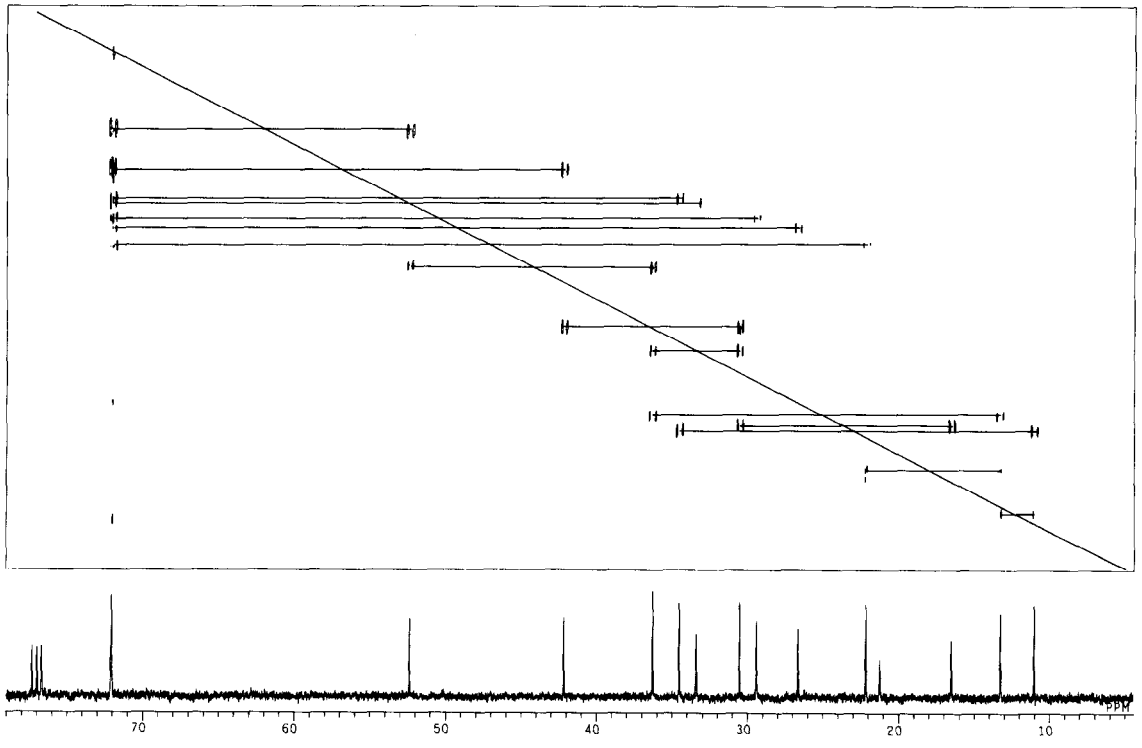

Fig. 2. ^{13}C shift correlation two-dimensional NMR spectrum of cyclokessyl acetate.

absolute configuration of cyclokessyl acetate to be established as depicted in formula **1**.

To the best of our knowledge, no natural and synthetic compound with this type of skeleton has been reported previously.

Although cyclokessyl acetate at 0.01-1 mg/ml in the culture medium showed no antihepatotoxic action in the carbon tetrachloride-induced cytotoxicity model system employing primary cultured rat hepatocytes, at 0.1 mg/ml in the culture medium, it did exhibit significant protective activity against D-galactosamine-produced liver damage in primary cultured rat hepatocytes.[8,9]

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