[Chem. Pharm. Bull. 17(8)1659—1663(1969)] UDC 547.597.04 : 576.8.098

# Biochemical Syntheses. IV.[1] Microbial Transformation of Kessane[2]

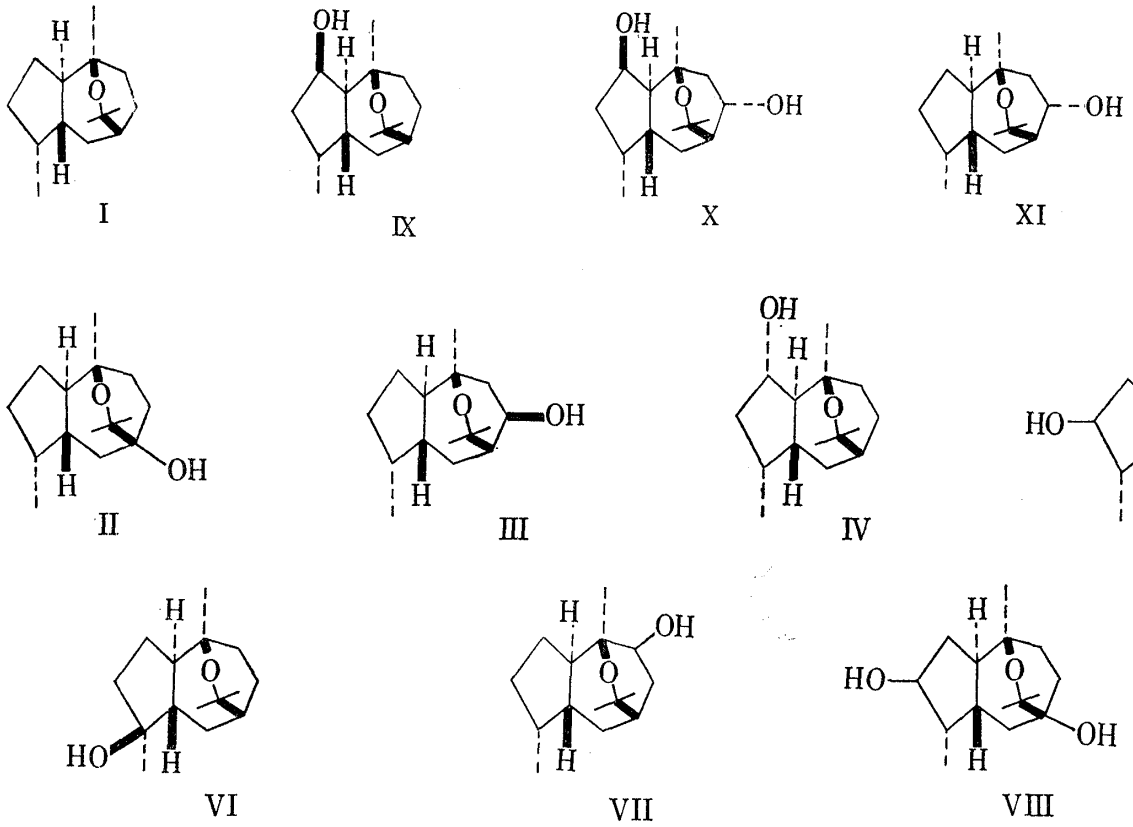
Hiroshi Hikino, Tomoaki Kohama, and Tsunematsu Takemoto

*Pharmaceutical Institute, Tohoku University*[3]

(Received February 13, 1969)

Fermentation of kessane, a constituent of valerian roots, with *Cunninghamella blakesleeana* has afforded seven oxygenated derivatives which have been concluded to be kessan-7-ol (II), 8-*epi*-kessanol (III), 2-*epi*-α-kessyl alcohol (IV), kessan-3ξ-ol (V), kessan-4β-ol (VI), kessan-9ξ-ol (VII), and kessane-3ξ,7-diol (VIII) on the basis of the chemical and physico-chemical properities.

Recently we[4] have carried out microbial transformation of α-kessyl alcohol (IX), a constituent of several different species of Japanese valerian,[5] and found that it reproduces the biochemical procedure (*i.e.* the selective hydroxylation) which actually takes place in the valerian plants giving kessyl glycol (X), another constituent of Japanese valerians.[5]




1) Part III: H. Hikino, S. Nabetani, and T. Takemoto, *Yakugaku Zasshi*, **89**, 809 (1969).
2) This paper is Part XXXIV in the series on Sesquiterpenoids. Preceding paper, Part XXXIII, H. Hikino, K. Aota, and T. Takemoto, *Chem. Pharm. Bull.* (Tokyo), **17**, 1390 (1969).
3) Location: *Aobayama, Sendai.*
4) H. Hikino, Y. Tokuoka, Y. Hikino, and T. Takemoto, *Tetrahedron*, **24**, 3147 (1968).
5) H. Hikino, Y. Hikino, H. Kato, Y. Takeshita, and T. Takemoto, *Yakugaku Zasshi*, **83**, 219 (1963); **89**, 117 (1969); H. Hikino, Y. Hikino, Y. Isurugi, and T. Takemoto, *ibid.*, **83**, 555 (1963); H. Hikino, Y. Hikino, Y. Takeshita, H. Kato, and T. Takemoto, *ibid.*, **85**, 179 (1965).

Two other sesquiterpenoids possessing the kessane skeleton, kessanol (XI) and 8-*epi*-kessanol (III), are known to exist in Nature.[6] These kessane derivatives must be biosynthesized by enzymatic hydroxylation of the common precursor kessane (I)[7] in the plants. In the hope that some of the natural kessane derivatives might be formed by fermentation of kessane (I) with microorganisms, present work was initiated.

After several screening tests with a number of microorganisms, *Cunninghamella blakesleeana* was selected as the representative. On incubation with the microbe, kessane (I) yielded seven products (II—VIII) which were separated by silica gel chromatography.

The least polar product (II) has the composition $C_{15}H_{26}O_2$ deduced from the molecular ion peak at *m/e* 238 in the mass spectrum. An infrared (IR) band at 3430 $cm^{-1}$ indicates that a hydroxyl group has been introduced into the kessane skeleton. The nuclear magnetic resonance (NMR) spectrum shows the presence of a secondary methyl (0.79 ppm) and three tertiary methyls on carbons bearing an ethereal oxygen (1.03, 1.17 and 1.20 ppm). Since no signal due to a carbinyl hydrogen is visible, the incorporated hydroxyl must be tertiary and, therefore, must be located at C–1, C–5, or C–7 in the kessane skeleton. If it were situated at C–1, the lowfield shift of the C–14 methyl signal by the hydroxyl group in the spatially close relationship should be observed, and if it were located at C–5, the downfield shift of the C–13 methyl signal should be found by a similar reason. In reality, however, the C–13 or C–14 methyl signal does not show such a lower-field displacement as compared with the corresponding signal of kessane (I). Consequently, the C–7 position for the location of the introduced hydroxyl group remains. This was supported by the following NMR evidence. Thus, the shifts between the methyl signals of the product (II) and kessane (I) which may be due to the contribution of the C–7 hydroxyl group are in agreement with those between the methyl signals of kessane-2$\beta$,7-diol and $\alpha$-kessyl alcohol, and those between the methyl signals of the acetate of the product (II) and kessane (I) which may be attributed to the contribution of the C–7 acetoxyl group are in accordance with those between the methyl signals of 2$\beta$,7-diacetoxykessane and $\alpha$-kessyl acetate (Table I and II). These results demonstrate that the product is kessan-7-ol.

Table I. Chemical Shifts of Methyl Protons (in $CCl_4$, ppm from TMS)

| Compounds | C–12 | C–13 | C–14 | C–15 |
|---|---|---|---|---|
| Kessane (I) | 1.20 | 1.20 | 0.78 | 1.02 |
| Kessan-7-ol (II) | 1.17 | 1.20 | 0.79 | 1.03 |
| 7-Acetoxykessane (XII) | 1.22 | 1.26 | 0.86 | 1.04 |
| $\alpha$-Kessyl alcohol (IX) | 1.19 | 1.22 | 0.77 | 1.29 |
| Kessane-2$\beta$,7-diol (XIII) | 1.22 | 1.22 | 0.79 | 1.33 |
| $\alpha$-Kessyl acetate (XIV) | 1.23 | 1.23 | 0.81 | 1.10 |
| 2$\beta$,7-Diacetoxykessane (XV) | 1.26 | 1.26 | 0.88 | 1.13 |

Table II. Contribution of C–7 Substituents on Methyl Chemical Shifts

| Substituents | C–12 | C–13 | C–14 | C–15 |
|---|---|---|---|---|
| Hydroxyl (II—I) | −0.03 | 0 | +0.01 | +0.01 |
| Hydroxyl (XIII—IX) | +0.03 | 0 | +0.02 | +0.04 |
| Acetoxyl (XII—I) | +0.02 | +0.06 | +0.08 | +0.02 |
| Acetoxyl (XV—XIV) | +0.03 | +0.03 | +0.07 | +0.03 |

The second product (III) was identified as the natural 8-*epi*-kessanol.

The third product (IV) was identified as 2-*epi*-$\alpha$-kessyl alcohol which has already been prepared from $\alpha$-kessyl ketone, kessan-2-one, by lithium aluminum hydride reduction.[2]

The fourth product (V) possesses the molecular formula $C_{15}H_{26}O_2$ as evidenced by the parent peak at *m/e* 238 in the mass spectrum. Introduction of a hydroxyl group was shown by an IR band at 3400 $cm^{-1}$. The NMR spectrum exhibits a methyl doublet (0.76 ppm) and three methyl singlets (0.99, 1.14 and 1.16 ppm). A 1H signal at *ca.* 3.9 ppm demonstrates the introduced hydroxyl group to be secondary. Chromic acid oxidation was then carried out to give a ketone which was revealed to have a carbonyl in a five-membered ring from the fact that it showed an IR band at 1745 $cm^{-1}$. Since it is not identical with kessan-2-one, it must be kessan-3-one. Consequently, the product (V) is concluded to be kessan-3-ol. However, the configuration of the hydroxyl group could not be established due to lack of the material.

The fifth product (VI) has the composition $C_{15}H_{26}O_2$. An IR band at 3350 $cm^{-1}$ indicates the formation of a hydroxyl group. The NMR spectrum shows four singlets at 1.00, 1.07, 1.20 and 1.20 ppm associated with methyl groups on oxygen-bearing carbons. This demonstrates the absence of a 3H doublet at 0.78 ppm attributed to the C-14 methyl protons in the spectrum of kessane (I), and instead the formation of the 3H singlet at 1.00 ppm. Since biological hydroxylation is known to proceed with the retention of the configuration, the fifth product (VI) is, therefore, concluded to be kessan-4$\beta$-ol.

The sixth product (VII) possesses the formula $C_{15}H_{26}O_2$ which indicates the presence of one oxygen atom more than in the parent substance (I), and this increment is corresponding to the incorporation of a hydroxyl group as evidenced by an IR band at 3430 $cm^{-1}$. The NMR spectrum shows a methyl doublet (0.77 ppm), three methyl singlets (1.01, 1.16 and 1.18 ppm) and a carbinyl multiplet ($\sim$4.2 ppm), demonstrating that the introduced hydroxyl is secondary. Chromic acid oxidation afforded a ketone whose IR spectrum exhibited a band at 1723 $cm^{-1}$ attributed to a carbonyl in a slightly strained six-membered ring, *i.e.*, the C ring in this case.[6] Therefore, the carbonyl is located at either C-8 or C-9. However, the former possibility can be excluded by the facts that the product (VII) and its oxidation product are not identical with kessanol (XI) or 8-*epi*-kessanol (XII) and kessan-8-one, respectively. Therefore, the oxidation product must be kessan-9-one. This conclusion was further confirmed by the solvent-induced shifts of the methyl signals in the NMR spectra of the oxidation product ($\Delta_{C_6H_6}^{CHCl_3}$ +0.05, +0.05, +0.34 and −0.23 ppm for the C-12, 13, 14 and 15 methyl protons) which indicate that the C-12, 13 and 14 methyl groups lie behind the reference plane and the C-15 methyl group is situated in front of the plane.[8] Further, the opticalrotatory dispersion (ORD) curve of the oxidation product shows a positive Cotton effect which is in agreement with the prediction from the octant diagram of kessan-9-one; kessan-3-one and kessan-6-one being expected to show negative Cotton effects.

The most polar product (VIII) has the composition $C_{15}H_{26}O_3$. The introduction of hydroxyls was evidenced by an IR band at 3430 $cm^{-1}$. The NMR spectrum shows a methyl doublet (0.84 ppm) and three methyl singlets (1.04, 1.20 and 1.20 ppm). Since the presence of a secondary hydroxyl group was indicated ($\sim$3.7 ppm), chromic acid oxidation was carried out to give a keto-alcohol whose IR spectrum disclosed the retention of a hydroxyl group (3450 $cm^{-1}$) and the formation of a cyclopentanone moiety (1745 $cm^{-1}$). The latter band indicates the introduced secondary hydroxyl group to be located at C-2 or C-3. The solvent-shifts of the NMR methyl signals of the ketol induced on passing from chloroform to benzene solution are +0.13, +0.07, +0.20 and +0.26 ppm for the C-12, C-13, C-14 and C-15 methyl protons, a fact which demonstrates that all the methyl groups are situated behind the carbonyl group. This finding shows the location at C-3 of the carbonyl group in the keto-alcohol and,

6) H. Hikino, Y. Hikino, Y. Takeshita, K. Shirata, and T. Takemoto, *Chem. Pharm. Bull.* (Tokyo), **11**, 952 (1963); H. Hikino, Y. Hikino, Y. Takeshita, K. Shirata, M. Ono, and T. Takemoto, *ibid.*, **15**, 324 (1967).
7) H. Hikino, Y. Hikino, Y. Takeshita, K. Shirata, and T. Takemoto, *Chem. Pharm. Bull.* (Tokyo), **11**, 547 (1963); *ibid.*, **15**, 321 (1967).
8) D.H. Williams and N.S. Bhacca, *Tetrahedron*, **21**, 2021 (1965).

consequently, of the secondary hydroxyl group in the original product (VIII). As has been evident from the above observations, the product (VIII) possesses another hydroxyl group which must be tertiary and, therefore, situated at C–1, C–5 or C–7. If the tertiary hydroxyl group were located at the C–1$\alpha$ or C–5$\beta$ position, there must be observed a lowfield shift of the C–14 or C–13 methyl signal in the NMR spectrum of the product (VIII) as opposed to those of kessane (I), as discussed above. However, no significant downfield displacement as expected was found in both signals, the line position of the C–14 methyl signal, slightly shifted lower-field (−0.06 ppm) as compared with that of the starting material kessane (I), being considered to be due to the C–3 hydroxyl group. This suggests the remaining hydroxyl group to be situated at C–7. Therefore, the product (VIII) is concluded to be kessane-3$\xi$,7-diol.

Among the metabolites described above, 8-*epi*-kessanol (III) is the only substance which has been found in the higher plant, valerian. It should be noted that a number of metabolites, which have not been discovered in higher plants, have been formed from kessane by a microbe, hydroxylases produced by the former being different from those induced by the latter.

## Experimental[9]

**Fermentation of Kessane with *Cunninghamella blakesleeana*——***Cunninghamella blakesleeana* was grown in a modified *Corticium* synthetic medium[4] (10 liter in one hundred 500 ml flasks) at 27° on a reciprocal shaker. After 3 days of incubation, kessane (I) (3.5 g) dissolved in EtOH (100 ml) was distributed equally among the hundred flasks. The fermentation was continued at 27° for 4 days. The culture broth then was extracted with AcOEt, and the extract evaporated to dryness. The residue (3.2 g) was repeatedly chromatographed over silica gel to give the following metabolites.

Kessan-7-ol (II) as a colorless oil. MS *m/e*: 238 ($M^+$). IR ($CCl_4$) $cm^{-1}$: 3640, 3430 (hydroxyl). NMR: Table I.

8-*epi*-Kessanol (III) as colorless needles (from light petroleum), mp 144.5—145°. IR (KBr) $cm^{-1}$: 3440 (hydroxyl). NMR: 3H d at 0.75 ($J$=6, C$\underline{H}_3$-CH$\langle$), three 3H s's at 1.05, 1.18, 1.31 (C$\underline{H}_3$-C$\langle$O–), 1H dd at 4.00 ($J$=8.5, 8.5, $\underline{H}$–C$\langle$OH). The identity with the natural 8-*epi*-kessanol was confirmed by the usual criteria.

2-*epi*-$\alpha$-Kessyl alcohol (IV) as colorless needles (from light petroleum), mp 106.5—107.5°. IR ($CCl_4$) $cm^{-1}$: 3430 (hydroxyl). NMR: 3H d at 0.91 ($J$=7, C$\underline{H}_3$–CH$\langle$), three 3H s's at 1.14, 1.21, 1.21 (C$\underline{H}_3$–C$\langle$O–), 1H ddd at 3.98 ($J$=6, 8.5, 8.5, $\underline{H}$–C$\langle$OH). Identification with 2-*epi*-$\alpha$-kessyl alcohol prepared from $\alpha$-kessyl ketone was carried out in the usual criteria.

Kessan-3$\xi$-ol (V) as a colorless oil. MS *m/e*: 238 ($M^+$). IR ($CCl_4$) $cm^{-1}$: 3400 (hydroxyl). NMR: 3H d at 0.76 ($J$=6, C$\underline{H}_3$–CH$\langle$), three 3H s's at 0.99, 1.14, 1.16, (C$\underline{H}_3$–C$\langle$O–), 1H m at *ca.* 3.9 ($\underline{H}$–C$\langle$OH).

Kessan-4$\beta$-ol (VI) as colorless needles (from light petroleum), mp 45—45.5°. MS *m/e*: 238 ($M^+$). IR ($CCl_4$) $cm^{-1}$: 3350 (hydroxyl). NMR: 3H s at 1.00 (C$\underline{H}_3$-C$\langle$OH), three 3H s's at 1.07, 1.20, 1.20 (C$\underline{H}_3$–C$\langle$O–).

Kessan-9$\xi$-ol (VII) as a colorless oil. MS *m/e*: 238 ($M^+$). IR ($CCl_4$) $cm^{-1}$: 3430 (hydroxyl). NMR ($CCl_4$): 3H d at 0.77 ($J$=7, C$\underline{H}_3$–CH$\langle$), three 3H s's at 1.01, 1.16, 1.18 (C$\underline{H}_3$–C$\langle$O–), 1H m at *ca.* 4.2 ($\underline{H}$–C$\langle$OH).

Kessane-3$\xi$,7-diol (VIII) as a colorless oil. MS *m/e*: 254 ($M^+$). IR ($CHCl_3$) $cm^{-1}$: 3430 (hydroxyl). NMR: 3H d at 0.84 ($J$=7, C$\underline{H}_3$–CH$\langle$), three 3H s's at 1.04, 1.20, 1.20 (C$\underline{H}_3$–C$\langle$O–), 1H m at *ca.* 3.7 ($\underline{H}$–C$\langle$OH).

**Acetylation of Kessan-7-ol**——Kessan-7-ol (II) (44 mg) in $Ac_2O$ (0.5 ml) was heated under reflux in the presence of AcONa (0.2 g) for 24 hr. Upon isolation in the usual manner, the product (45 mg) was chromatographed over silica gel (5 g) to afford 7-acetoxykessane as a colorless oil. IR ($CCl_4$) $cm^{-1}$: 1735, 1234 (acetoxyl). NMR: Table I.

**Chromic Acid Oxidation of Kessan-3$\xi$-ol**——To kessan-3$\xi$-ol (V) (10 mg) in ether (3 ml) was added $Na_2Cr_2O_7 \cdot 2H_2O$ (100 mg) and $H_2SO_4$ (60 mg) in water (0.6 ml), and the mixture was stirred at room temperature for 30 min. Isolation in the usual manner gave kessan-3-one as a colorless oil. IR ($CCl_4$) $cm^{-1}$: 1745 (cyclopentanone).

**Chromic Acid Oxidation of Kessan-9$\xi$-ol**——Kessan-9$\xi$-ol (VII) (20 mg) was oxidized in similar manner described above to afford kessan-9-one as colorless needles. mp 151—152°. ORD ($c$=0.0579, MeOH): $[\phi]_{320}^{peak}$ +7490, $[\phi]_{278}^{trough}$ −5100. IR ($CCl_4$) $cm^{-1}$: 1723 (carbonyl in a strained six-membered ring). NMR ($CHCl_3$): 3H d at 0.90 ($J$=7, C$\underline{H}_3$–CH$\langle$), three 3H s's at 1.29, 1.29, 1.40 (C$\underline{H}_3$–C$\langle$O–), NMR ($C_6H_6$): 3H d at 0.56 ($J$=7, C$\underline{H}_3$–CH$\langle$), three 3H s's at 1.24, 1.24, 1.63(C$H_3$–C$\langle$O–).

9) NMR spectra were measured in $CCl_4$ solution at 60 MHz unless otherwise specified. Chemical shifts are given in ppm downfield from internal $Me_4Si$, and coupling constants ($J$) in Hz. Abbreviations: s=singlet, d=doublet, m=multiplet and dd=doublet of doublets.

**Chromic Acid Oxidation of Kessane-3ξ,7-diol**——Kessane-3ξ,7-diol (VIII) (15 mg) was subjected to chromic acid oxidation as described above to yield kessan-3-on-7-ol as a colorless oil, IR ($CCl_4$) $cm^{-1}$: 3450 (hydroxyl), 1745 (cyclopentanone), NMR ($CHCl_3$): 3H d at 0.98 ($J=7$, C<u>H</u>$_3$–CH$\langle$), three 3H s's at 1.14, 1.31, 1.32 (C<u>H</u>$_3$–C$\langle$O–), NMR ($C_6H_6$): 3H d at 0.78 ($J=7$, C<u>H</u>$_3$–CH$\langle$), three 3H s's at 0.88, 1.18, 1.25 (C<u>H</u>$_3$–C$\langle$O–).

**Acknowledgement** Thanks are due to Research Laboratory, Yoshitomi Pharmaceutical Co., Ltd., for the mass spectra, to Analytical Laboratory, Department of Chemistry, this University, for the NMR spectra, and to Analytical Laboratory, this Institute, for the NMR spectra.