Tetrahedron Vol 24, pp 3147 to 3152 Pergamon Press 1968 Printed in Great Britain

BIOCHEMICAL SYNTHESES—I

MICROBIAL TRANSFORMATION OF α-KESSYL ALCOHOL TO KESSYL GLYCOL AND KESSANE-2β,7-DIOL*

H. HIKINO, Y. TOKUOKA, Y. HIKINO and T. TAKEMOTO

Pharmaceutical Institute, School of Medicine, Tohoku University
Kita-4-bancho, Sendai, Japan

(*Received in Japan* 20 *September* 1967; *accepted for publication* 24 *October* 1967)

Abstract The microbiological attack of *Cunninghamella blakesleeana*, *Corticium sasakii*, *Corticium centrifugum*, and *Streptomyces aurofaciens* on α-kessyl alcohol (I), a constituent of valerian roots, has resulted in the selective hydroxylation at two positions (8α and 7) yielding kessyl glycol (II), another constituent of valerian, and kessane-2β,7-diol (III).

α-KESSYL alcohol and kessyl glycol are constituents of several different species of Japanese valerian.[1-3] We have recently elucidated their stereostructures as shown in formulas I and II, respectively.[4] Chemical transformation of kessyl glycol to α-kessyl alcohol has already been achieved by Ukita.[5] On the other hand, the selective 8α-hydroxylation of α-kessyl alcohol to kessyl glycol by a chemical procedure is extremely difficult. As this transformation is effected exclusively by enzymes in the plants, microorganisms were investigated in the hope that enzymatic hydroxylation of α-kessyl alcohol to kessyl glycol, could be achieved. Although the hydroxylation of steroids by microorganisms has ample precedence, microbiological transformation of terpenoids has been reported in only a few instances. During our survey on the

TABLE 1. RELATIVE AMOUNT OF EACH PRODUCT DURING FERMENTATION (%)*

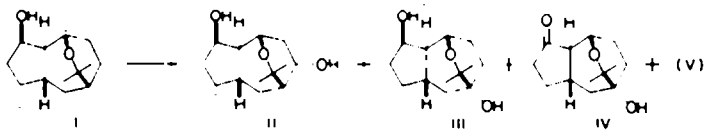
Species of microbes	*Cunninghamella blakesleeana*		*Corticium sasakii*		*Corticium centrifugum*		*Streptomyces aureofaciens*	
Products	Kessyl glycol (II)[b]	Kessane-2β,7-diol (III)	Kessyl glycol (II)[b]	Kessane-2β,7-diol (III)	Kessyl glycol (II)[b]	Kessane-2β,7-diol (III)[c]	Kessyl glycol (II)[b]	Kessane-2β,7-diol (III)
Duration of cultivation (days)								
2	34	66	50	50	45	55	87	13
4	34	66	50	50	45	55	86	14
6	35	65	50	50	45	55	87	13
8	36	64	51	49	44	56	87	13
16	35	65	51	49	46	54	88	12

[a] This was calculated from vapor phase chromatogram peak area.
[b] This includes a trace amount of the ketol (IV).
[c] This includes a trace amount of the ketol (V).

* This paper is Part XXII in the series on Sesquiterpenoids. Part XXI, H. Hikino, N. Suzuki and T. Takemoto, *Chem. Pharm. Bull.* in press.

biooxidation of α-kessyl alcohol, we discovered a number of microorganisms which oxidize it selectively.

As shown in Table 1, fermentation of α-kessyl alcohol (I) with *Cunninghamella blakesleeana* readily yields two major products (II and III), together with a minor product (IV). Although α-kessyl alcohol disappears in the medium during an early period of cultivation, the components accumulate until the substrate is exhausted and are not metabolized further but retain almost constant relative proportions. This was later confirmed by incubation of each major product (II or III), isolated in a pure condition, which yielded practically no metabolites the starting substrate (II or III) being recovered.



I II III IV

The isolation of three components was readily accomplished by alumina chromatography. The major, more polar product (II) was identified as the natural kessyl glycol. Another major, less polar product (III) has the composition $C_{15}H_{26}O_3$. The IR spectrum ($CHCl_3$) shows OH absorption at 3640 and 3460 cm^{-1} but no CO absorption. In accordance with the expectance that the product (III) would have the kessane skeleton, NMR signals indicate the presence of a secondary Me and three Me's on carbons bearing an oxygen. Provided that the product (III) has the kessane skeleton, the molecular formula and the IR spectral properties suggest it to be a di-hydroxy derivative. The NMR spectrum exhibits a one-proton triplet (J = 4 c/s) at 3·97 ppm whose line position and splitting pattern agree with those of 2α-hydrogen in 2β-hydroxylated derivatives of kessane, e.g., α-kessyl alcohol (I) and kessyl glycol (II), suggesting that the 2β-OH group in α-kessyl alcohol is still retained in the diol (III). Since there are no other NMR signals in a region where hydrogens on carbons attached to OH's could be anticipated to occur, the second OH group must be tertiary. The assignment that the diol (III) has a secondary and a tertiary OH was verified by acetylation with acetic anhydride in pyridine at room temperature to give the monoacetate (VI) whose IR (KBr) and NMR spectra reveal the presence of a tertiary OH (3520 and 3420 cm^{-1}) as well as a secondary acetoxyl grouping (1728 and 1234 cm^{-1}, and 3H singlet at 1·99 ppm and 1H triplet at 4·92 ppm). Further, on oxidation with chromic acid the diol (III) was converted into the keto-alcohol (IV) which shows in the IR ($CHCl_3$) a band at 1730 cm^{-1} attributed to a cyclopentanone moiety and gives a very similar optical rotatory dispersion curve (a + 130) to that (a + 148[4]) of α-kessyl ketone (IX), also confirming the secondary OH group in the diol (III) to be situated at C-2. The remaining OH group judging from its known tertiary nature, must be located at C-1, C-5, or C-7 in the kessane skeleton. Treatment of the ketol (IV) with alkaline alumina afforded the isomerized ketol (VII), which exhibits an IR band at 1730 cm^{-1} associated with a cyclopentanone, and shows a negative Cotton curve (a −151) similar to that (a −194[4]) of isokessyl ketone (X). This transformation can be explained by the epimerization at C-1, adjacent to the C-2 CO group, a fact which is compatible with the transformation from α-kessyl ketone (IX) to isokessyl ketone (X) by the same treatment. This observation excludes the possibility that the tertiary OH group is

oriented at C-1 or C-5. Since, if it were located at C-1, the epimerization would be highly improbable under the conditions employed, and if it were situated at C-5, the epimerization would occur with concomitant dehydration to give a cyclopentenone system. Further, dehydration of the diol monoacetate (VI) with phosphorus oxychloride in pyridine was attempted recovering the starting monoacetate (VI). This is probably due to the OH group being situated at a bridgehead position. Therefore, the second tertiary OH group is oriented at C-7. This assignment was verified by the following NMR evidence. Since biological hydroxylation is known to proceed with retention of configuration, either the C-14 or C-13 Me signal of the diol (III) should suffer a downward shift, if the OH group has been introduced to C-1 or C-5, respectively; both Me and OH being oriented in a spatially close relation in each case.[6] Furthermore, the downward shift should be relieved more or less on acetylation of the OH group.[6] In reality, however, the C-14 or C-13 Me signals of the diol (III) and the diol monoacetate (VI) show no downfield shifts as compared with the corresponding signals of α-kessyl alcohol (I) and α-kessyl acetate, respectively, and consequently, either signal of the diol diacetate (VIII), prepared from the diol (III) on refluxing with acetic anhydride in the presence of sodium acetate, also exhibits no acetylation shift in contrast to the corresponding signal of diol monoacetate (VI) (Table 2). These results indicate that the product (III) is kessane-2β,7-diol.

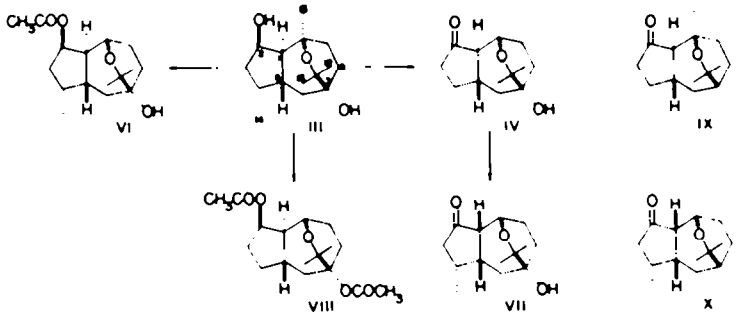


TABLE 2. CHEMICAL SHIFTS OF METHYL PROTONS (IN CCl_4, PPM FROM TMS)

Compounds	C-12	C-13	C-15	C-14
α-Kessyl alcohol (I)	1·19	1·22	1·29	0·77
Kessane-2β,7-diol (III)	1·22	1·22	1·33	0·79
α-Kessyl acetate	1·23	1·23	1·10	0·81
2β-Acetoxy-kessan-7-ol (VI)	1·21	1·21	1·10	0·82
2β,7-Diacetoxy-kessane (VIII)	1·26	1·26	1·13	0·88

The minor product (IV) has the molecular formula $C_{15}H_{24}O_3$ and the spectral data appears to be identical with kessan-2-on-7-ol, which was previously prepared from

kessane-2β,7-diol (III) by chromic acid oxidation. Direct comparison of both ketols confirmed the identity. The yield of the ketol IV was very small in every period of cultivation.

Fermentation of α-kessyl alcohol (I) with *Corticium sasakii* yielded the following proportion of products—45% kessyl glycol (II), 55% kessane-2β,7-diol (III) and a trace of kessan-2-on-7-ol (IV).

On cultivation with *Corticium centrifugum*, α-kessyl alcohol (I) produced kessyl glycol (II; 50% yield), kessane-2β,7-diol (III; 50% yield) and kessan-2-on-7-ol (IV; trace), together with a trace amount of a ketol (V) whose IR spectrum ($CHCl_3$) shows the presence of a CO (1720 cm^{-1}) and a OH group (3630 and 3450 cm^{-1}). Insufficient amounts of the ketol (V) were available for full characterization.

Incubation of α-kessyl alcohol (I) with *Streptomyces aureofaciens* afforded mainly kessyl glycol (II; 87% yield) besides kessane-2β,7-diol (III; 13% yield) and kessan-2-on-7-ol (IV; trace).

Almost constant distribution patterns of the products during cultivation were also obtained in each case (Table 1).

The transformation of α-kessyl alcohol (I) by each of the four microorganisms has shown that the positions 7 and 8α are involved in the hydroxylation, although the degree of specificity reaction regarding the two positions depends upon the species. Thus, the actual biochemical procedure (i.e., the selective hydroxylation of kessyl alcohol (I)) which takes place in the valerian plants can be reproduced by microorganisms.

In addition, α-kessyl alcohol and the derivatives obtained were screened for central nervous system and cardiovascular properties. α-Kessyl alcohol exhibits weak motor stimulatory activity at a dose of 300 mg/kg (*per os*) in mice, but no activity at a dose of 100 mg/kg. No effect was observed with kessyl glycol and kessane-2β,7-diol.

EXPERIMENTAL

M.ps are uncorrected. The rotations were taken in $CHCl_3$ solution. NMR spectra were determined at 60 Mc/s except as noted to the contrary. Chemical shifts are expressed in ppm from TMS as internal standard and coupling constants (J) in c/s.

General procedure for fermentation. A 500 ml volume flask was charged with medium containing $NaNO_3$ (0·3 g), K_2HPO_4 (0·1 g), $FeCl_2 \cdot 4H_2O$ (0·05 g), KCl (0·05 g), $MgSO_4 \cdot 7H_2O$ (0·05 g), soluble starch (3 g), vitamin solution* (1 ml), and H_2O (100 ml). The pH of the medium was adjusted to 6·8 with N NaOH, and the vessel and medium were sterilized at 120° for 20 min. After cooling to room temp, the flask was inoculated with mycelia. The culture was shaken at 27° for a period of 6 days. A soln of a substrate (30 mg) in EtOH (1 ml) was added to each flask and the fermentation was continued at 27° for 6 days.

The filtrate of the culture broth was then extracted with AcOEt and the extract was evaporated to give a fermentation product.

Fermentation of α-kessyl alcohol with Cunninghamella blakesleeana. The harvested fermentation product, obtained from α-kessyl alcohol by action of *Cunninghamella blakesleeana*, was chromatographed on alumina.

Elution with benzene and crystallization from acetone yielded *kessan-2-on-7-ol* (IV) as colorless needles, m.p. 174–177°, MS *m/e* (relative intensity): 252 (2, parent peak), 194 (30), 176 (25), 137 (100, base peak),

* The vitamin solution contains biotin (0·2 mg), folic acid (0·2 mg), *p*-aminobenzoic acid (20 mg), flavin mononucleotide (20 mg), calcium panthothenate (40 mg), nicotinic acid (40 mg), pyridoxine hydrochloride (40 mg), thiamine hydrochloride (40 mg), inositol (200 mg), and H_2O (1 l.).

109 (35), 97 (30), 95 (41), 43 (26), IR ($CHCl_3$) cm^{-1}: 3640, 3460 (hydroxyl), 1731 (cyclopentanone), NMR ($CDCl_3$, 100 Mc·s*): doublet (3H) at 0·92 (J = 7 c/s, $C\underline{H}_3$ CH<), singlet (6H) at 1·28 (CH_3 C(O)<), singlet (3H) at 1·40 ($C\underline{H}_3$—C(O)<). The identity with the ketol (IV) derived from kessane-2β,7-diol (III) (*vide infra*) was confirmed in the usual criteria.

Successive elution with benzene gave kessyl glycol (II) as a colorless oil, IR ($CHCl_3$) cm^{-1}: 3630, 3450 (OH), identified by behavior on TLC and VPC, and IR spectrum.

Further elution with benzene–AcOEt (1:1) and distillation under reduced press afforded *kessane-2β,-7-diol* (III) as colorless needles, m.p. 58–60°, $[\alpha]_D$ −29·1° (c = 3·0), MS m/e (relative intensity): 254 (1, parent peak), 178 (39), 161 (30), 145 (26), 142 (100, base peak), 123 (30), 121 (42), 120 (30), 109 (27), 99 (26), 97 (29), 93 (24), 69 (26), 43 (26), IR ($CHCl_3$) cm^{-1}: 3640, 3460 (OH), NMR ($CHCl_3$): doublet (3H) at 0·82 (J = 7 c/s, CH_3—CH<), singlet (6 H) at 1·22 ($C\underline{H}_3$—C(O)—), singlet (3H) at 1·32 ($C\underline{H}_3$—C(O)—), triplet (1H) at 3·97 (J = 4 c/s, $-CH_2$ $C\underline{H}$(OH)—CH<).

Partial acetylation of kessane-2β,7-diol. Compound III (50 mg) in pyridine (2 ml) and Ac_2O (0·4 ml) was set aside at room temp for 2 days. Upon isolation in the usual manner, the product was chromatographed over silica gel (3 g). Elution with benzene–AcOEt (10:1) and crystallization from AcOEt gave *2β-acetoxy-kessan-7-ol* (VI) as colorless plates (27 mg), m.p. 118–120°, $[\alpha]_D$ −99·0° (c = 2·0), IR (KBr) cm^{-1}: 3520, 3420 (OH), 1728, 1234 (acetoxyl), NMR (CCl_4): doublet (3H) at 0·85 (J = 7 c/s, $C\underline{H}_3$-CH<), singlet (3H) at 1·11 (CH_3-C(O)-), singlet (6H) at 1·21 ($C\underline{H}_3$—C(O)—), singlet (3H) at 1·99 ($C\underline{H}_3$ CO-O-), triplet (1H) at 4·92 (J = 5 c/s, CH_2 $C\underline{H}$(OCOCH$_3$)-CH<).

Oxidation of kessane-2β,7-diol with chromic acid. Compound III (60 mg) in ether (6 ml) was stirred with $Na_2Cr_2O_7 \cdot 2H_2O$ (120 mg) in H_2SO_4 (0·4 ml) and water (1·6 ml) at room temp for 3 hr. Isolation followed by crystallization from AcOEt afforded IV as colorless needles (39 mg), m.p. 188–189·5°, ORD (c = 0·0743, MeOH): $[\phi]_{326}^{peak}$ +7430, $[\phi]_{324}^{trough}$ +7330, $[\phi]_{317}^{peak}$ +7730, $[\phi]_{280}^{trough}$ −5300, MS m/e (relative intensity): 252 (2, parent peak), 194 (25), 176 (22), 137 (100, base peak), 109 (27), 97 (25), 95 (33), 43 (27), IR ($CHCl_3$) cm^{-1}: 3640, 3480 (OH), 1730 (cyclopentanone), NMR ($CDCl_3$): doublet (3H) at 0·92 (J = 7 c/s, $C\underline{H}_3$ CH<), singlet (6H) at 1·29 ($C\underline{H}_3$ C(O)-), singlet (3H) at 1·40 ($C\underline{H}_3$ C(O)-).

Epimerization of kessan-2-on-7-ol with alumina. Compound IV (20 mg) in MeOH (1 ml) was adsorbed on a column of alkaline allumina (5 g), and left overnight. Elution with MeOH and distillation under diminished press gave *isokessan-2-on-7-ol* (VII) as a colorless oil, ORD (c = 0·0734, MeOH): $[\phi]_{325}^{trough}$ −7560, $[\phi]_{320}^{peak}$ −7350, $[\phi]_{315}^{trough}$ −8030 $[\phi]_{276}^{peak}$ +7040, IR ($CHCl_3$) cm^{-1}: 3640, 3480 (OH), 1730 (cyclopentanone), NMR ($CDCl_3$): doublet (3H) at 1·06 (J = 7 c/s, $C\underline{H}_3$ CH<), singlet (6H) at 1·03 ($C\underline{H}_3$ C(O)-), singlet (3H) at 1·32 ($C\underline{H}_3$ -C(O)-).

Attempted dehydration of the monoacetate with phosphorus oxychloride. The monoacetate VI (12 mg) in pyridine (0·5 ml) was treated with $POCl_3$ (0·05 ml) at room temp for 3 days. Ether extraction gave a product (8 mg) which was identified as recovered VI by TLC and IR spectrum.

Complete acetylation of kessane-2β,7-diol. Compound III (18 mg) and AcONa (20 mg) in Ac_2O (0·5 ml) were heated under reflux for 5 hr. After isolation, the product was crystallized from light petroleum to yield *2β,7-diacetoxy-kessane* (VIII) as colorless plates (18 mg), IR (KBr) cm^{-1}: 1720, 1240 (acetoxyl), NMR (CCl_4): doublet (3H) at 0·88 (J = 7 c/s, $C\underline{H}_3$ CH<), singlet (3H) at 1·13 ($C\underline{H}_3$ C(O)—), singlet (6H) at 1·26 ($C\underline{H}_3$-C(O)-), singlet (3H) at 1·89 ($C\underline{H}_3$ CO-O-), singlet (3H) at 1·98 (CH_3-CO-O-), triplet (1H) at 4·93 (J = 5 c/s, CH_2 $C\underline{H}$(OCOCH$_3$)-CH<).

Fermentation of kessyl glycol with Cunninghamella blakesleeana. The harvested fermentation product, obtained from kessyl glycol by action of *Cunninghamella blakesleeana*, was shown by TLC and VPC to consist of the starting substrate (II).

* We thank Japan Electron Optics Laboratory Co. Ltd., who obtained the spectrum by iterative addition of 16 time runs through a resonance accumulator.

Fermentation of kessan-2β,7-diol with Cunninghamella blakesleeana. The harvested fermentation product, obtained from kessane-2β,7-diol by action of *Cunninghamella blakesleeana*, was revealed by TLC and VPC to be essentially composed of the starting substrate (III) only.

Fermentation of α-kessyl alcohol with Corticium sasakii. The harvested fermentation product, obtained from α-kessyl alcohol by action of *Corticium sasakii*, was chromatographed over alumina. Elution with benzene gave IV, identified by TLC and VPC. Successive elution with the same solvent yielded II, identified by TLC, VPC and IR. Elution with benzene–AcOEt (1:1) afforded III, identified by TLC, VPC and IR.

Fermentation of α-kessyl alcohol with Corticium centrifugum. The harvested fermentation product, obtained from α-kessyl alcohol by action of *Corticium centrifugum*, was subjected to chromatography on alumina. Elution with benzene gave IV, identified by TLC and VPC. Further elution with benzene furnished II, identified by TLC, VPC and IR. Elution with benzene–AcOEt (1:1) gave III, identified by TLC, VPC and IR. Elution with benzene- AcOEt (1:2) afforded V as a colorless oil, IR ($CHCl_3$) cm^{-1}: 3630, 3450 (OH), 1720 (CO in 5-membered or strained 6-membered ring).

Fermentation of α-kessyl alcohol with Streptomyces aureofaciens. The harvested fermentation product, obtained from α-kessyl alcohol by action of *Streptomyces aureofaciens*, was submitted to alumina chromatography. Elution with benzene gave IV, identified by TLC and VPC. Successive elution with the same solvent yielded II, identified by TLC, VPC and IR. Elution with benzene–AcOEt (1:1) furnished III, identified by TLC, VPC and IR.

Acknowledgements We express our deep gratitude to Prof. N. Ishida and Dr. K. Kumagai of this School, for helpful advice on fermentation. The cultures of the microbes used were supplied by Institute for Fermentation, Osaka, to whom thanks are due. We are also grateful to Research Laboratories, Takeda Chemical Industries Ltd., for the mass spectra, Analytical Laboratories, this Institute, for the NMR spectra, and Bristol Laboratories, for the biological screening.

REFERENCES

[1] H. Hikino, Y. Hikino, H. Kato, Y. Takeshita and T. Takemoto, *Yakugaku Zasshi* **83**, 219 (1963).
[2] H. Hikino, Y. Hikino, Y. Takeshita, Y. Isurugi and T. Takemoto, *Ibid.* **83**, 555 (1963).
[3] H. Hikino, Y. Hikino, Y. Takeshita, H. Kato and T. Takemoto, *Ibid.* **85**, 179 (1965).
[4] S. Itô, M. Kodama, T. Nozoe, H. Hikino, Y. Hikino, Y. Takeshita and T. Takemoto, *Tetrahedron Letters* 1787 (1963); *Tetrahedron* **23**, 553 (1967).
[5] T. Ukita, *Yakugaku Zasshi* **65**, 458 (1945).
[6] Y. Kawazoe, Y. Sato, M. Natsume, H. Hasegawa, T. Okamoto and K. Tsuda, *Chem. Pharm. Bull.* **10**, 338 (1962).