crease in free fraction of tolmetin (present at 26.7 μg/ml) from 0.29 ± 0.02% (Mean ± *SD*) to 1.0 ± 0.12%. It appears that factors other than the metabolite itself must have contributed to the increase in tolmetin free fraction observed by these investigators.

The fact that I does not readily displace tolmetin from plasma protein binding sites has important implications in the understanding of tolmetin disposition. Since the metabolite circulates at concentrations lower than those of tolmetin in healthy subjects (1, 4) and arthritic patients (5), no effect of I on tolmetin disposition would be anticipated based on the data presented in Table I. In anephric patients receiving tolmetin, I accumulates in plasma to concentrations often >10-fold those of tolmetin[8]. Some displacement of tolmetin by the metabolite in these patients might be expected. However, since uremic plasma has reduced binding affinity for tolmetin (6), it is difficult to assess the additional influence of high circulating metabolite concentrations on tolmetin disposition in the uremic patient.

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Hydroxyisolongifolaldehyde: A New Metabolite of (+)-Longifolene in Rabbits

Keyphrases □ Longifolene—hydroxyisolongifolaldehyde, a new metabolite in rabbits □ Metabolites—hydroxyisolongifolaldehyde from longifolene in rabbits □ Hydroxyisolongifolaldehyde—a new metabolite of longifolene in rabbits

To the Editor:

In spite of daily usage of terpenoid-containing plant products such as fruits, drinks, tobacco, *etc.*, the metabolic fate of terpenoids is essentially unknown. The aim of this study is to clarify terpenoid biotransformation in mammalian biochemistry from the toxicological point of view. This will provide guidance for the effective usage of natural products and for preparation of starting materials for organic synthesis.

Wild rabbits damage the forests in Japan by feeding on the artificially planted young *Chamaecyparis obtusa*, an important tree used commercially in Japan. This Japanese cypress contains longifolene (I) as a major sesquiterpene hydrocarbon. The metabolism of α-pinene, camphene, and other related terpenoids in rabbits was studied previously (1–7).

Longifolene (36 g), $[\alpha]_D$ = +39.3°, in chloroform, was administered to rabbits and the metabolites were isolated according to the method described previously. The neutral crude alcohols (3.7 g), were acetylated and purified to afford the main acetate (II) (17% for total acetates), which gave the following spectral data: mass spectrum: *m/z* 278 ($C_{17}H_{26}O_3$); 2700 and 1710 cm^{-1}; and a small doublet (J = 1.5 Hz) at 9.88 ppm of an aldehyde group. It was hydrolyzed by potassium carbonate in methanol and on silica gel chromatography gave pure metabolic alcohol (III). The parent ion of III, *m/z* 236, means a molecular formula $C_{15}H_{24}O_2$ showing that two oxygen atoms were introduced into longifolene. These oxygen atoms are based on a hydroxyl group (3450 cm^{-1}) and an aldehyde group (2700 and 1720 cm^{-1}; 9.87 ppm). The signals at 3.46 and 3.31 ppm (AB quartet) of this alcohol shifted 0.5 ppm upfield compared with those of acetate, suggesting the presence of a primary hydroxyl group. The olefin group of longifolene was lost in this alcohol. Thus, the change of this *exo*-methylene group to an aldehyde or primary alcohol was expected. The positions of newly introduced groups were determined on the basis of IR, ^{1}H-NMR, and ^{13}C-NMR spectra.

First, the aldehyde proton signal of III split into a doublet (J = 2.0 Hz) indicating the presence of an adjacent 7-H proton. By this splitting, it was concluded that the position of the aldehyde group is not at C-12, C-14, and C-15 but at C-7. The 7-H configuration was determined on the basis of a coupling constant. Provided that 7-H is in an α-configuration, the coupling constants of this proton would be anticipated to be $J_{7,1}$ = 1.3–2.6 Hz and $J_{7,8}$ = ~0.5 Hz. However, if 7-H is in a β-configuration, these values would be expected to be: $J_{7,8}$ = 3.6–5.0 Hz, $J_{7,9}$ ~1.2 Hz, and $J_{7,11}$ = 0.5 Hz. In fact, $J_{7,8}$ in III was observed as 3.5 Hz meaning 7-H has the β-configuration. Thus, the configuration of this aldehyde group was determined to be in the C-7-*endo*-form, which is stable in isolongifolaldehyde (8).

Second, in the IR spectra of the metabolized alcohol and

Table I—^{13}C-NMR Chemical Shifts in Longifolene and Hydroxyisolongifolaldehyde

	Longifolene (I)	Hydroxyisolongifolaldehyde (III)
C-1	62.2	56.3
C-2	33.6	37.8
C-3	36.5	34.2
C-4	21.2	21.0
C-5	43.4	44.2
C-6	44.1	43.0
C-7	168.0	59.9
C-8	48.0	40.8
C-9	29.8	25.8
C-10	25.5	23.0
C-11	45.2	47.0
C-12	30.1[a]	26.0[a]
C-13	99.0	206.4
C-14	30.6[a]	22.6[a]
C-15	30.6[a]	72.6

[a] Assignments may be reversed in each compound.

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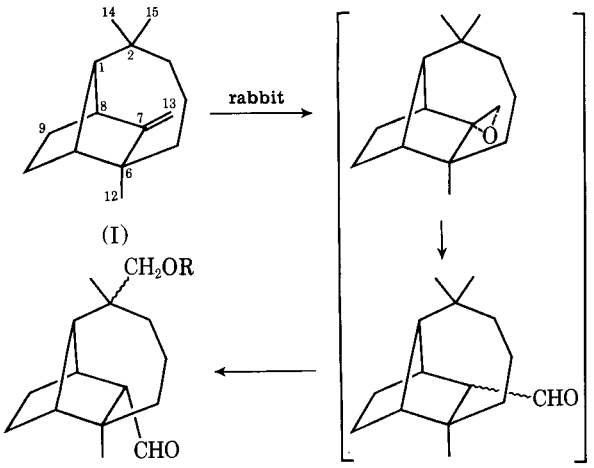

(II) R = CH_3CO
(III) R = H

Scheme I

its acetate, the absorption of the *gem*-dimethyl group, which appeared at 1355 and 1370 cm^{-1} in longifolene, was lost and instead a new single absorption (1360 cm^{-1}) appeared in this region. This suggests that one of the *gem*-dimethyl groups at C-2 was hydroxylated producing a primary alcohol. Furthermore, in the ^{13}C-NMR of III, the downfield shift of the C-2 carbon and the upfield shifts at C-1, C-3, and C-14 or C-15 were observed when compared with longifolene (Table I)[1].

The determination of which methyl (C-14 or C-15) was hydroxylated remains to be established. It is well known that the orientation of the C-7 substituent group in longifolene changes easily from the *exo*- to *endo*-form. However, such isomerization of the C-7 aldehyde during isolation processes was not found by TLC, GLC, or IR. Therefore, longifolene would be metabolized in rabbits as follows: (*a*) attack on the *exo*-methylene group from the *endo*-face to form its epoxide; and (*b*) isomerization of the epoxide to a stable *endo*-aldehyde. Thus, the metabolic route of longifolene would be written as in Scheme I.

In the metabolism of compounds having *exo*-methylene groups such as myrcene (2), camphene (3), and camphene epoxide (4), their glycols were obtained and their formation can be explained through epoxides. The reason for not finding longifolene-7,13-glycol can be attributed to the less stable longifolene-7,13-epoxide.

Terpenic aldehydes are biotransformed from camphor (dog) (9) and fenchone (rabbit) (10). Introduction of an aldehyde group in biotransformation is remarkable, and this isolongifolaldehyde may have some biological activity which is found in other sesquiterpene aldehydes such as polygodial. The hydroxylation of *gem*-dimethyl groups producing a primary alcohol has been reported on the compounds having three- (1, 2), four- (7), five- (10), and six- (11) membered rings in mammals. In this study, hydroxylation of the *gem*-dimethyl group on the seven-membered ring was newly added.

[1] The complete analysis of ^{13}C-NMR spectrum of longifolene was based on the comparison with that of longifolol, which was kindly furnished by Dr. K. Tori, Shionogi Research Laboratory, Shionogi & Co., Ltd., Japan.

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