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# The Structure of Isousnic Acid with Reference to "Isodihydrousnic Acid" derived from Dihydrousnic Acid[1]

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Isousnic acid (IIIb), a structural isomer of usnic acid, was isolated from lichens of *Cladonia* spp. The so-called "Isodihydrousnic acid" obtained by the thermal rearrangement of dihydrousnic acid has been discussed in comparison with isodihydrousnic acid (XII) prepared by catalytic hydrogenation of isousnic acid.

In the previous papers[3,4] we established that usnic acid (IIIa) is biosynthesized in lichens by phenol oxidative coupling of methylphloroacetophenone (I) followed by cyclization to complete the ether linkage. However, there are another three possibilities for the second step cyclization to give the structural isomers, IIIb, IIIc and IIId.

During the biosynthetical study of usnic acid using a lichen, *Cladonia mitis* Sandst. we found an unknown spot accompanying that of usnic acid on the thin-layer chromatogram of the ethereal extracts. This compound has been proved to be one of the assumed structural isomers of usnic acid as being formulated as IIIb, and now is named isousnic acid on which the present paper mainly concerns.

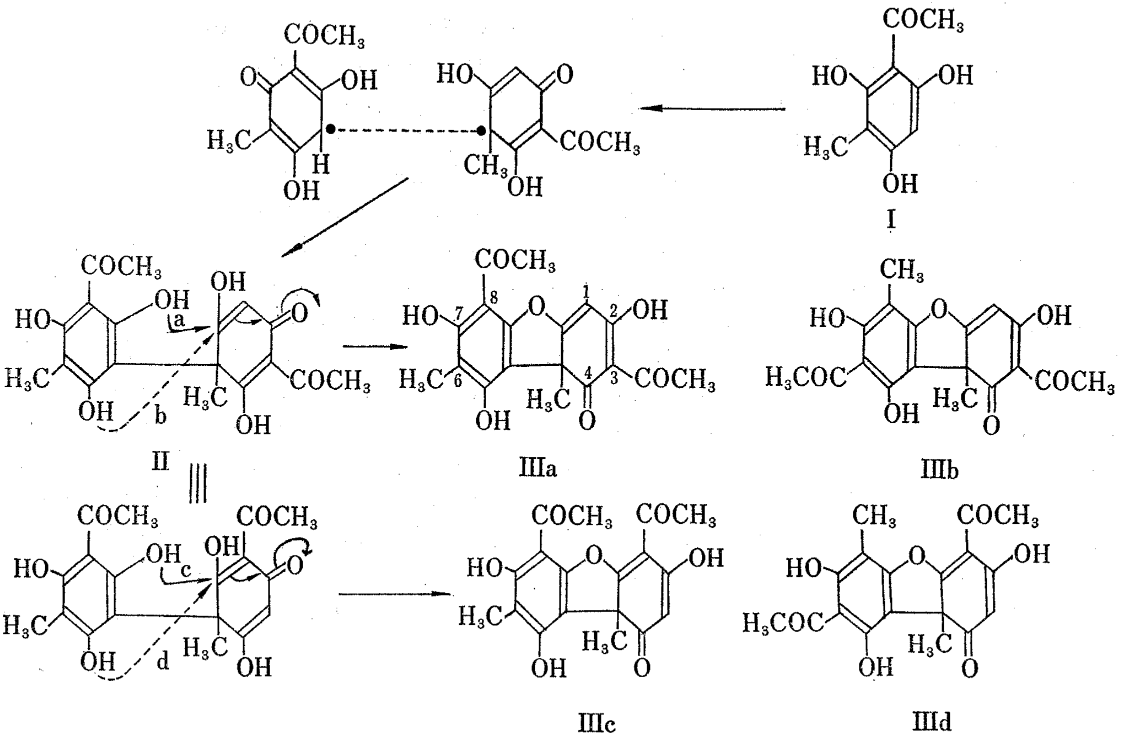

Chart 1

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[1] The preliminary report of this work was published in *Tetrahedron Letters*, 1967 4867.

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[3] H. Taguchi, U. Sankawa and S. Shibata, *Chem. Pharm. Bull.* (Tokyo), 17, 2054 (1969).

[4] H. Taguchi, U. Sankawa and S. Shibata, *Chem. Pharm. Bull.* (Tokyo), 17, 2061 (1969).

(+) Isousnic acid (IIIb), $C_{18}H_{16}O_7$, yellow prisms, mp 150—152°, $[\alpha]_D^{31}$ +457° (in $CHCl_3$, $c=0.8$) obtained from the lichen by the method described in the experimental part showed a close similarity with usnic acid[5] in the UV and IR spectral and chemical properties. It gave a deep blue green colouration with Ehrlich's reagent (*p*-dimethylaminobenzaldehyde and HCl) in contrast with a blue colouration of usnic acid. The structural isomeric relation with usnic acid was also suggested by a high incorporation (0.6—0.9%) of methylphloroacetophenone ($CO^{14}CH_3$) into isousnic acid in feeding experiment.

The NMR spectrum of isousnic acid resembles that of usnic acid except the following few points. The acetyl groups of isousnic acid give two separated signals while those of usnic acid show a duplicated singlet. The signals of hydroxyls at 5 and 7-positions of isousnic acid appear in lower field than those of usnic acid (Table I).

Table I

| | Isousnic acid (IIIb) | Usnic acid (IIa) |
|---|---|---|
| UV $\lambda_{max}^{EtOH}$ (log $\varepsilon$) | 232 (4.42), 282 (4.47), 327 (3.91) | 220 (4.44), 290 (4.45), 325 (3.85) infl. |
| NMR in $CDCl_3$ ($\delta$, TMS=0) at 60 Mc | | |
| Angular C$\underline{H}_3$ | 1.75 (3H, s) | 1.75 (3H, s) |
| Arom. C$\underline{H}_3$ | 2.10 (3H, s) | 2.10 (3H, s) |
| -COC$\underline{H}_3$ | 2.67 (3H, s), 2.78 (3H, s) | 2.68 (6H, s) |
| Arom. H | 5.95 (H, s) | 5.98 (H, s) |
| -OH($C_5$) | 11.30 (H, s) | 11.03 (H, s) |
| -OH($C_7$) | 14.36 (H, s) | 13.29 (H, s) |
| -OH($C_2$) | 18.70 (H, s) | 18.84 (H, s) |

Accordingly, isousnic acid might have a different disposition of acetyl and methyl groupings in A ring in comparing with those of usnic acid.

This has been confirmed by the ozonolysis of O-diacetylisousnic acid (IV), $C_{22}H_{20}O_9$, mp 110—112° (resolidifies at 120—122° and remelts at 181—183.5°), forming 4,6-diacetoxy-5-acetyl-3,7-dimethylcoumaran-2-one (V), $C_{16}H_{16}O_7$, mp 124—126°, whose UV and IR spectra are very similar to those of the ozonolytic product of O-diacetylusnic acid (VI), which was formulated as 4,6-diacetoxy-7-acetyl-3,5-dimethylcoumaran-2-one (VII), $C_{16}H_{16}O_7$, mp 130—132°[6] (Table II). On deacetylation with $H_2SO_4$, the product (V) afforded 7-acetyl-4,6-

Table II. UV, IR and NMR Spectra of 4,6-Diacetoxy-5-acetyl-3,7-dimethylcoumaran-2-one-(V) and 4,6-Diacetoxy-7-acetyl-3,5-dimethylcoumaran-2-one-(VII)

| | V | VII |
|---|---|---|
| UV $\lambda_{max}^{EtOH}$ (log $\varepsilon$) | 253 (3.86), 330 (3.07) | 261 (3.88), 330 (3.44) |
| IR $\nu_{max}^{CHCl_3}$ cm$^{-1}$ | | |
| $\beta$-$\gamma$-Unsaturated $\gamma$-lactone | 1818 | 1823 |
| Arom. -O-$COCH_3$ | 1775 | 1770 (1775 sh) |
| Arom. $COCH_3$ | 1699 | 1690 |
| NMR (in $CDCl_3$, $\delta$, TMS=O) at 60 Mc | | |
| $-C\langle^{H}_{\underline{CH}_3}$ | 1.51 (d, $J$=7.5 cps) | 1.52 (d, $J$=7.5 cps) |
| Arom. $CH_3$ | 2.11 (3H, s) | 1.97 (3H, s) |
| Arom. O-$COCH_3$ | 2.30 (6H, s) | 2.33 (3H, s), 2.38 (3H, s) |
| Arom. $COCH_3$ | 2.44 (3H, s) | 2.60 (3H, s) |
| $-C\langle^{\underline{H}}_{CH_3}$ | 3.77 (1H, q, $J$=7.5 cps) | 3.77 (1H, q, $J$=7.5 cps) |

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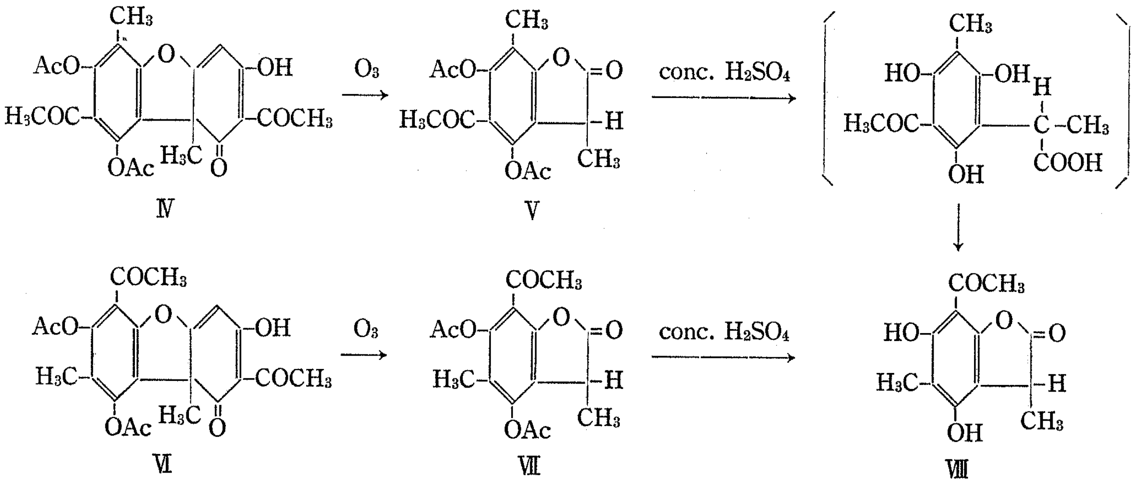
5) (+) Usnic acid, $C_{18}H_{16}O_7$: yellow prisms, mp 203—204°, $[\alpha]_D$ +492° (in $CHCl_3$).

6) C. Schöpf and F. Ross, *Ann. Chem.*, **546**, 1 (1941).

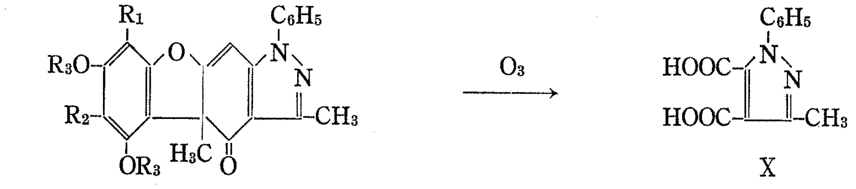


Ac=-COCH₃

Chart 2

dihydroxy-3,5-dimethylcoumaran-2-one (VIII) which is identical with the deacetylated product of VII. A rearrangement of lactone ring of V occurs in the deacetylation process.

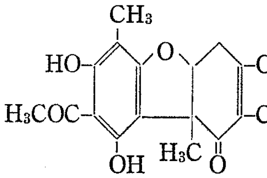
The structure of ring C of isousnic acid which was suggested as being same with that of usnic acid by the spectral analysis was established by the ozonolysis of isousnic acid anhydrophenylhydrazone (XI), $C_{24}H_{20}O_5 1/2C_2H_5OH$, mp 191—192.5°, obtaining 3-methyl-1-phenylpyrazole-4,5-dicarboxylic acid (X), which had been prepared by Barton, *et al.*[7] from O-diacetylusnic acid anhydrophenylhydrazone (IX).



IX : $R_1$=$COCH_3$, $R_2$=$CH_3$, $R_3$=$COCH_3$
XI : $R_1$=$CH_3$, $R_2$=$COCH_3$, $R_3$=H

Chart 3

Thus the structure of isousnic acid (IIIb) has finally been established.



XII

Catalytic hydrogenetion of (+) isousnic acid with Pd-black in tetrahydrofuran afforded (+) isodihydrousnic acid (XII), $C_{18}H_{18}O_7$, mp 109—115°, $[\alpha]_D^{23}$ +157° (in dioxane) which gave very similar UV and IR spectra with those of (−) dihydrousnic acid, $C_{18}H_{18}O_7$, mp 147°, $[\alpha]_D$ −85°, prepared from (+) usnic acid.

On the other hand, "(+) isodihydrousnic acid", $C_{18}H_{18}O_7$, mp 128°, $[\alpha]_D$=+47.5°, was reported by Shibata, Takahashi and Tanaka[8] as a thermal rearrangement product of (+) dihydrousnic acid. Since "isodihydrousnic acid" gave double shifting NMR signals, it was explained by a tautomeric equilibrium state of the compound in solution.[9] However, (+) dihydroisousnic acid prepared by catalytic hydrogenation of (+) isousnic acid showed no double shifting in the NMR spectrum.

As (+) isodihydrousnic acid is readily converted into (−) dihydrousnic acid even by the recrystallization process, "isodihydrousnic acid" must be contaminated with dihydrousnic

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7) D.H.R. Barton and T. Brunn, *J. Chem. Soc.*, **1953**, 603.
8) S. Shibata, K. Takahashi, and Y. Tanaka, *Pharm. Bull.*, **4**, 65 (1956).
9) J. Shoji, *Chem. Pharm. Bull.* (Tokyo), **10**, 483 (1962).

acid. This has now been confirmed by the thin–layer chromatography of the former sample of "isodihydrousnic acid" giving two distinct spots.

Recently, Nuno[10] examined 1050 specimens of 57 *Cladonia* species and found that 8 species including *Cladonia submitis* Evans, *Cl.pleurota* Schaer, and *Cl.sylvatica* Harm., which belong mainly to Cladina subgenus contain isousnic acid along with usnic acid. A lichen of *Sphaero phorus* spp. collected in New Zealand contains only isousnic acid and not usnic acid.

## Experimental

**Isolation of (+) Isousnic Acid**——*Cladonia mitis* Sandst. was extracted with ether, and the extracts were chromatographed on silica gel impregnated with 0.5N oxalic acid eluting with benzene–hexane (1:1) to separate two yellow bands. From the lower yellow band yellow crystals were obtained on evaporation of the solvent followed by addition of small amount of MeOH. (+) Isousnic acid (IIIb) was thus isolated in yellow prisms on recrystallization with benzene–EtOH (yield 0.3%).

From the upper yellow band, (+) usnic acid was isolated (yield 1.8%). (+) Isousnic acid, mp 150—152° $[\alpha]_D^{31}$ +475° (in $CHCl_3$, $c=0.8$), is soluble in benzene, $CHCl_3$ and ether and sparingly s oluble in EtOH and MeOH. It gave a redbrown colour with 5% $FeCl_3$ and a deep blue green colour with Ehrlich's reagent. *Anal.* Calcd. for $C_{18}H_{16}O_7$: C, 62.79; H, 4.68. Found: C, 62.73; H, 4.59. UV $\lambda_{max}^{EtOH}$ m$\mu$ (log $\varepsilon$): 232 (4.42), 282 (4.47), 327 (3.91).

**(+) Diacetylisousnic Acid (IV)**——(+) Isousnic acid (500 mg) was dissolved in $Ac_2O$ (5 ml) added with 2—3 drops of conc. $H_2SO_4$ and allowed to stand overnight. The precipitates formed on pouring into ice water were recrystallized from MeOH to obtain pale yellow cubes, mp 110—112°. $[\alpha]_D^{21}$ +289° (in dioxane, $c=0.048$) (resolidify at 120—122°, and remelt at 181—183.5°. $[\alpha]_{700\text{-}300}^{21} \pm 0$ (in dioxane, $c=0.048$)). *Anal.* Calcd. for $C_{22}H_{20}O_9$: C, 61.68; H, 4.71. Found: C, 61.77; H, 4.85. UV $\lambda_{max}^{EtOH}$ m$\mu$ (log $\varepsilon$): 264 (4.29), 305 (3.74) inflex. NMR (in $CDCl_3$ at 60 Mc) $\delta$. ppm. 1.84 (3H, s, angular $CH_3$), 2.14 (3H, s, arom. $CH_3$), 2.33 (3H, s), 2.40 (3H, s) [2 × arom. $OCOC\underline{H}_3$], 2.46 (3H, s), 2.53 (3H, s) [2 × arom. $COC\underline{H}_3$], 5.87 (H, s, arom. $\underline{H}$), 18.25 (H, s, $C_2$–OH). IR $\nu_{max}$ (KBr) $cm^{-1}$: 1780, 1770, 1695, 1663, 1620, 1607.

**Ozonolysis of (+) O-Diacetylisousnic Acid (IV) (Formation of 4,6-Diacetoxy-5-acetyl-3,7-dimethylcoumaran-2-one (V))**——O-Diacetylisousnic acid (IV) (500 mg) was dissolved in $CCl_4$ (20 ml) and ozonized at 0°. After 30 min the yellow colour of solution changed to orange and after 2 hr turned into colourless to separate the colourless ozonide precipitate. Added with 5 ml of water, the reaction mixture was refluxed on a boiling water bath for 15 min to decompose the ozonide. Acidic substance was removed by shaking with 1% $NaHCO_3$ solution, and the solvent was evaporated. The residue was recrystallized from EtOH to give colurless cubes, 4,6-diacetoxy-5-acetyl-3,7-dimethylcoumaran-2-one (V), mp 124—126°, $[\alpha]_D=0°$ (in $CHCl_3$). *Anal.* Calcd. for $C_{16}H_{16}O_7$: C, 60.00; H, 5.01. Found: C, 59.95; H, 5.28.

A remarkable depression of melting point (mp 98—106°) was observed on admixture with 4,6-diacetoxy-7-acetyl-3,5-dimethylcoumaran-2-one (VII) $C_{16}H_{16}O_7$, mp 130—132°, prepared from O-diacetylusnic acid by the same procedure.

**7-Acetyl-4,6-dihydroxy-3,5-dimethylcoumaran-2-one (VIII)**——4,6-Diacetoxy-5-acetyl-3,7-dimethylcoumaran-2-one (V) (100 mg) was dissolved in conc. $H_2SO_4$ (3 ml) and, the reaction mixture which was allowed to stand for 10 min was poured into ice water. The precipitates formed were filtered and recrystallized from MeOH to obtain pale yellow needles, mp 220—222°. *Anal.* Calcd. for $C_{12}H_{12}O_5$: C, 60.91; H, 5.02. Found: C, 61.01; H, 5.12. UV $\lambda_{max}^{EtOH}$ m$\mu$ (log $\varepsilon$): 239 (4.04), 280 (4.18), 325 (3.81). IR (KBr) $cm^{-1}$: 3380 (OH), 1787 ($\beta,\gamma$-unsaturated $\gamma$-lactone), 1635—1620 (chelated C=O and aromatic). NMR (in DMSO) $\delta$ (ppm): 1.47 (3H, d. $J=7.5$ cps), 1.99 (3H, s, arom. $C\underline{H}_3$), 2.60 (3H, s, arom–$COC\underline{H}_3$), 3.90 (1H, q. $J=7.5$ cps), 11.39 (1H, s, $C_{(4)}$–OH), 13.31 (1H, s, $C_{(6)}$–OH).

It was identified with 7-acetyl-4,6-dihydroxy-3,5-dimethyl-coumaran-2-one (VIII), $C_{12}H_{12}O_5$, mp 224—226°, on mixed fusion, and by comparison of IR (KBr) spectra and thin–layer chromatograms (on silica gel G impregnated with oxalic acid using acetone–benzene (1:9) as the solvent).

**Isousnic Acid Anhydrophenylhydrazone (XI)**——A mixture of isousnic acid (200 mg) and phenylhydrazine hydrochloride (100 mg) in EtOH (5 ml) was refluxed for 14 hr. On cooling a yellow crystalline product was separated and recrystallized from EtOH to form yellow cubes, mp 191—192.5° (yield: 150 mg). *Anal.* Calcd. for $C_{24}H_{20}O_5N_2 \cdot \frac{1}{2}C_2H_5OH$: C, 68.21; H, 5.35; N, 6.37. Found: C, 68.33; H, 5.35; N, 6.77. UV $\lambda_{max}^{EtOH}$ m$\mu$ (log $\varepsilon$): 228 (4.53), 290 (4.46), 340 (3.87). IR (KBr) $cm^{-1}$: 1683, 1630, 1600, 1515. NMR (in $CDCl_3$) $\delta$ (ppm): 1.81 (3H, s. angular $C\underline{H}_3$), 2.06 (3H, s. arom. $C\underline{H}_3$), 2.60 (3H, s. $-N=\overset{|}{C}-C\underline{H}_3$), 2.79 (3H, s. arom. $C\underline{H}_3$), 4.24 (H, s. arom. H), 7.55 (5H, s. $-C_6\underline{H}_5$), 11.38 (1H, s. O$\underline{H}$), 14.30 (1H, s. O$\underline{H}$); $\frac{1}{2}C_2H_5OH$ (1.25 (1.5H, t. $J=8$); 3.74 (1H, q. $J=8$)).

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[10] M. Nuno, *Journ. Jap. Bot.*, 43, 359 (1968).

**3-Methyl-1-phenylpyrazole-4,5-dicarboxylic Acid (X) formed by Ozonolysis of Isousnic Acid Anhydrophenylhydrazone (XI)**——Ozonised air was passed through a solution of isousnic acid anhydrophenylhydrazone (XI) (500 mg) in $CCl_4$ (30 ml) under ice-cooling. After 20—30 min the colour of solution changed from yellow to orange red, and then turned into yellow separating finally colourless ozonide. Ethanol (5 ml) was added to the reaction mixture which was refluxed to obtain an orange coloured solution. From the reaction mixture acidic portion was extracted with 1% $NaHCO_3$ solution. On acidification with 1N $H_2SO_4$ the product was extracted with ether. A brown resinous residue obtained by evaporation was added with KOH–MeOH to form K salt, which was washed with MeOH and dissolved in water. The aqueous solution was acidified and extracted with ether to obtain colourless crystalline substance which was recrystallized from benzene, mp 189—191° (decomp.). It was identified by a mixed fusion and comparison of IR and thin–layer chromatograms with 3-methyl-1-phenylpyrazole-4,5-dicarboxylic acid (X), mp 193.5—195° (decomp.) which was prepared from usnic acid by the same procedure. UV $\lambda_{\max}^{\mathrm{EtOH}}$ mμ (log ε): 253 (11.600). NMR (in $d_6$-DMSO) δ (ppm): 2.56 (3H, s. C$\underline{H}_3$), 7.51 (5H, s, arom. $\underline{H}$).

**Isodihydrousnic Acid (XII)**——Isousnic acid (IIIb) (200 mg) dissolved in tetrahydrofuran (50 ml) was hydrogenated catalytically using Pd-black (250 mg). After 2 hr, the solvent was evaporated and the residue was recrystallized from MeOH to obtain colourless crystals, mp 109—115°, $[\alpha]_D^{23}$ +157 (in dioxane, $c=0.38$). *Anal.* Calcd. for $C_{18}H_{18}O_7$: C, 62.42; H, 5.24. Found: C, 62.69; H, 5.37. UV $\lambda_{\max}^{\mathrm{EtOH}}$ mμ (log ε): 228 (4.26), 286 (4.41), 325 (3.58) inflex.

**Incorporation of Methylphloroacetophenone (-$CO^{14}CH_3$) (I) into Isousnic Acid (IIIb)**——Fresh thalli of *Cladonia mitis* Sandst. (5 g) was suspended in Czapek–Dox medium (30 ml) in a Sakaguchi flask (500 ml). Methylphloroacetophenone ($CO^{14}CH_3$) (I) sp. activity $9.7\times10^8$ dpm/mM) dissolved in EtOH (1 ml) was added to the above medium and the mixture was shaken for 3 days under illumination of light (100 w/100 volt from 1 m distance).

The lichen thalli washed with water and dried were extracted to isolate isousnic acid (IIIb) and usnic acid (IIIa). The radioactivity was measured by liquid scintillation counter (Packard) using toluene scintillator (PPO 0.4% POPOP 0.01% in toluene). The observed value was corrected by the standard curve prepared using benzoic acid–$^{14}C$ as the standard and usnic acid as the quencher.

TABLE III

| | Methylphloroacetophenone administered Mci/5 g lichen | Isousnic acid sp. activity (dpm/mM) | Isousnic acid Incorp. ratio (%) | Usnic acid sp. activity (dpm/mM) | Usnic acid Incorp. ratio (%) |
|---|---|---|---|---|---|
| Exp. 1 | 1.47 | $8.6\times10^5$ | 0.6 | $4.8\times10^5$ | 3.5 |
| Exp. 2 | 1.47 | $9.1\times10^5$ | 0.9 | $4.8\times10^5$ | 2.7 |
| Exp. 3 | 0.5 | $1.7\times10^5$ | 0.68 | $3.6\times10^5$ | 7.5 |

**Acknowledgement** We wish to thank Prof. Emeritus Y. Asahina and Miss M. Nuno for their lichenological advices and supply of their data of microchemical survey work on lichens.