The equilibrium, however, is not present in this reaction, and acidification of alkaline solution of I gave a compound of mp 193°, $C_{11}H_{18}N_4O_2 \cdot 2HCl$ (C, 42.73; H, 6.52; N, 17.63; Cl, 23.08), deformyldesthiothiamine (V) easily.

It is quite natural that I has no catalytic ability (strongly positive test observed in Mizuhara's model acetoin test is mainly due to its degradation product, γ-aceto-γ-hydroxypropyl alcohol,[5] and no growth stimulating abilities to Kl. apiculata IFO 0630 or L. fermenti 36 (ATCC 9338) (The data will be published elsewhere) is probably due to its facile hydrolysis to IV in culture medium since the pK_a of I is too small to protect it from the ring opening in the experimental conditions.

The NMR spectrum of I in D_2O gave the chemical shift for 2 hydrogen at 0.21 which is consistent with that of 3,4-dimethyloxazole[17] and exchanged more rapidly with D than thiamine at pH 6.

The greater electronegativity of oxygen compared to sulfur results in both the nucleophylic attack at C-2 and deprotonization to III more easily than thiamine and the absence of the equilibrium for the reaction I to IV undoubtedly tend to push the reaction to IV. I should thus be inactive as a coenzyme inspite of its higher acidity of carbon 2 than thiamine.

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UDC 581.14.08 : 582.572.4 : 547.94.05

Lycoricidinol and Lycoricidine, New Plant-growth Regulators in the Bulbs of *Lycoris radiata* Herb.

Methanol extract of the bulbs of *Lycoris radiata* Herb. has strong growth-inhibiting actions on Avena straight growth test and rice seedling test.[1] This prompted us to isolate the active principle . The methanol extract was purified with the method outlined in Fig. 1, and two plant growth inhibitors, lycoricidinol and lycoricidine, were isolated.

In this communication,[2] we wish to report the establishment of the structures and biological activities of these two compounds.

Physical properties of the active substances are as follows:

Lycoricidinol (1), $C_{14}H_{13}O_7N$, has no sharp melting point, begins to color about 200°, and decomposes slowly above 216°. UV λ_{max}^{EtOH} mμ (ε): 252 (25600), 303 (6400), 330 (sh.); IR ν_{max}^{KBr} cm^{-1}:

1) The action had been found by Yo Isogai and detailed data will be published elsewhere.
2) In this work, molecular formulae of the compounds were determined by elemental analyses and/or mass spectrometry. All the melting points were measured on Yanagimoto micromelting point measuring apparatus and are uncorrected. Tetramethyl silane was used as the internal standard in NMR determination.

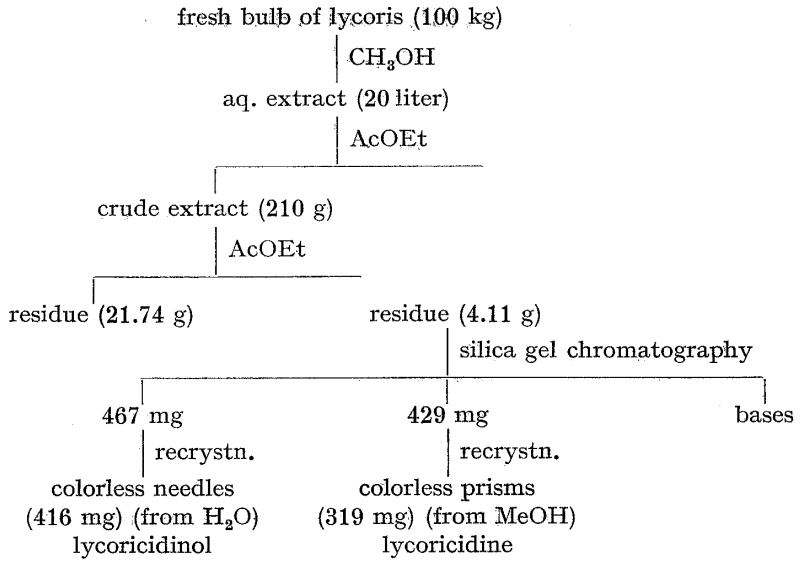


Fig. 1. Extraction and Purification

2400—3600 (OH), 1670 (amidic carbonyl group); NMR (d_6–DMSO) δ: 3.5—4.2 (complex, 4H), 4.5—5.3 (broad, 3H)[3] 6.0 (singlet, 2H), 6.1 (broad multiplet, 1H), 6.8 (singlet, 1H), 7.8 (broad singlet, 1H),[3] 13.2 (singlet, 1H).[3]

Lycoricidine (**2**), $C_{14}H_{13}O_6N$, mp 214.5—215.5° (decomp.); UV λ_{max}^{EtOH} mμ (ε): 241 (21300), 300 (5000), 325 (sh.); IR ν_{max}^{KBr} cm^{-1}: 2400—3600 (OH), 1660 (amidic carbonyl group); NMR (d_5–pyridine)δ: 4.6—5.15 (complex, 4H), 4.71 (singlet, 1H),[3] 5.95 (singlet, 2H), 6.53 (broad multiplet, 1H), 7.15 (singlet, 1H), 7.86 (singlet, 1H), 8.22 (broad singlet, 1H).[3]

Lycoricidinol (**1**) is easily soluble in aqueous sodium hydroxide solution and gives a deep green–violet color with ferric chloride. This indicates the presence of a phenolic hydroxyl group in **1**. On the other hand, **2** does not show such a character. In the infrared spectra, **1** and **2** show the bands assignable to carbonyl and hydroxyl groups. Ultraviolet spectra of **1** and **2** show similar absorption but the red shift in **1** can be attributed to the presence of a phenolic hydroxyl group. The significant difference in their NMR spectra is the presence of a signal of a chelated phenolic hydroxyl group (13.2, singlet) in **1** instead of an aromatic proton (7.86, singlet) in **2**. In the NMR spectra of **1** and **2**, the signals at 6.0 δ and 5.95 δ were assigned to a methylenedioxy group. Its presence was also confirmed by the sulfuric acid-chromotropic acid test.

When treated with acetic anhydride in pyridine, **1** gave a triacetate (**3**), mp

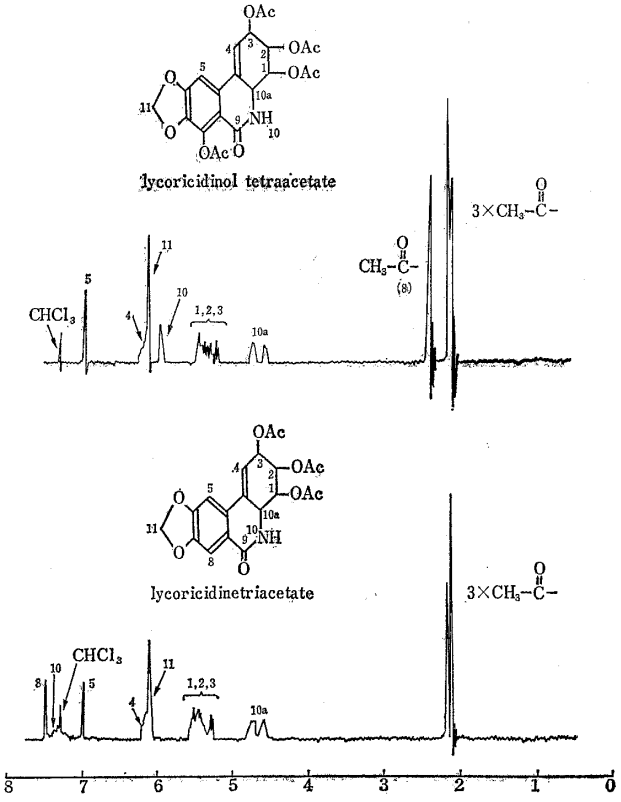
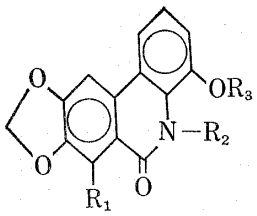


Fig. 2. NMR in $CDCl_3$ (TMS)

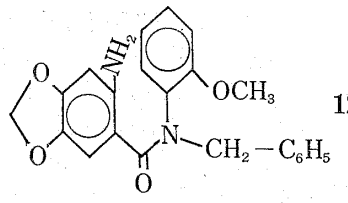
3) The signals disappeared on adding heavy water.

200—201°, and a tetraacetate (**4**), mp 229—231°, which were hydrolyzed to **1** in mild conditions. Ferric chloride test is possitive in **3** but negative in **4**. Therefore, the triacetate (**3**) has a phenolic hydroxyl group resistant to acetylation. Further, in the NMR spectrum of **3**, the signal of a chelated phenolic hydroxyl group is still observed (12.4 δ in $CDCl_3$). Acetylation of **2** gives only the triacetate (**5**), mp 201—202°. The acetates, **3**, **4** and **5**, give very similar NMR charts in $CDCl_3$, and the signals of three protons adjacent to acetyloxy group appear at 5.10—5.50, 5.10—5.50, and 5.15—5.50 δ, respectively. The NMR spectra of **4** and **5** are shown in Fig. 2. Lycoricidinol requires two moles of HIO_4 for oxidation and, therefore, in the three alcoholic hydroxyl groups are vicinal to each other in **1** and **2**.

On catalytic hydrogenation **1** and **2** take up one mole of hydrogen, giving dihydro compounds, (**6**) (mp 167—169°) and (**7**) (mp 258—261° (decomp.)), respectively. **6**, UV λ_{max}^{EtOH} mμ (ε): 234 (22100), 279 (7200), 310 (sh.). **7** UV λ_{max}^{EtOH} mμ (ε): 221 (25800), 260 (4400), 270 (sh.), 302 (5500). The marked blue shift in the UV spectra of the dihydro compounds clearly indicated that the hydrogenated double bond is conjugated with the aromatic ring. The signal of one proton at 6.1 δ in **1** disappears in **6**, so that the hydrogenated double bond is trisubstituted.



8 : R_1=OH, R_2=R_3=H
9 : R_1=R_2=R_3=H
10 : R_1=R_2=H, R_3=CH_3
11 : R_1=H, R_2=$CH_2-C_6H_5$, R_3=CH_3



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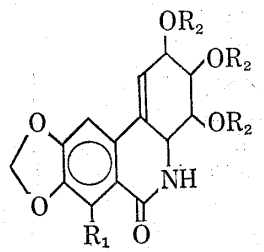
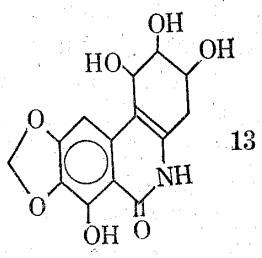
When **1** and **2** are treated with methanolic hydrochloric acid or dehydrated on palladium–charcoal catalyst, arolycoricidinol, mp 293—295°, and arolycoricidine, mp 281—283°, are respectively obtained, eliminating two molecules of water. Their UV spectra are similar to that of N–methyl–7,8–methylenedioxyphenanthridone or anhydrolycorine lactam.[4] This suggests the structures (**8**) and (**9**), respectively, for arolycoricidinol and arolycoricidine because ABC type aromatic ring system was indicated by their NMR spectra. Arolycoricidine was methylated to arolycoricidine methyl ether (**10**), mp 292—300° (decomp.), and further **10** was converted to N–benzylated product (**11**), mp 175—176°, with sodium hydride and benzyl chloride. The product (**11**) is identical with the authentic sample obtained from Pschorr cyclization of **12** in IR spectrum, thin layer chromatography, and mixed melting point. Thus the structure of arolycoricidine was definitely established.



1 : R_1=OH, R_2=H
2 : R_1=R_2=H
3 : R_1=OH, R_2=Ac
4 : R_1=OAc, R_2=Ac
5 : R_1=H, R_2=Ac



13

To satisfy the formation of the aro–compounds, the presence of three vicinal hydroxyl groups, and the other chemical and physical properties, the structures of lycoricidinol and

4) H. Kondo, K. Takeda, and K. Kodera, *Itsuu Kenkyusho Nempo*, No. 5, 14 (1954).

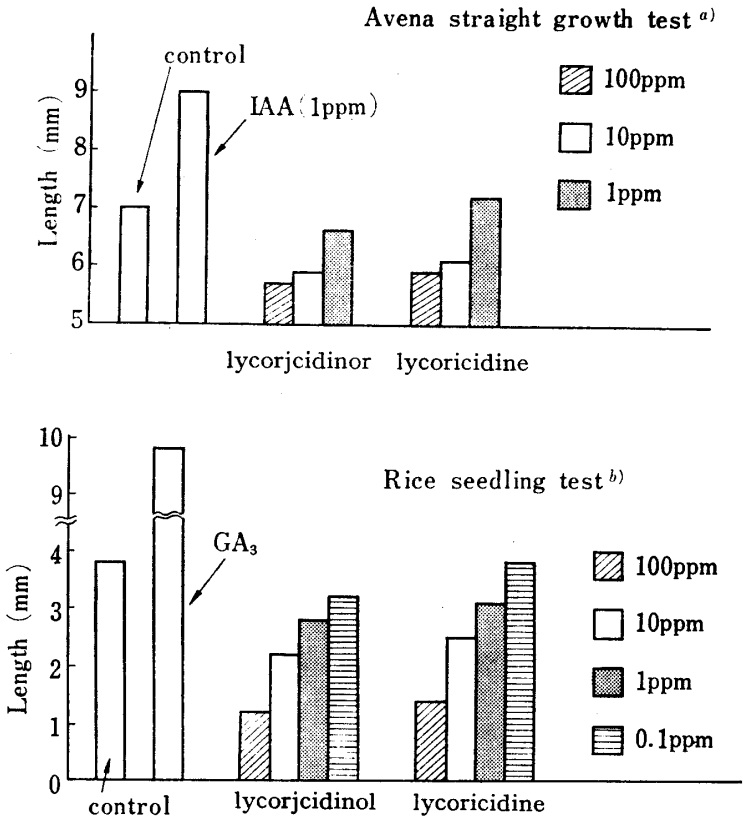


Fig. 3. Effects of Lycoricidinol and Lycoricidine

a) average length of 15 coleoptiles of *Avena sativa* var. Victory strain
b) average length of 20 second leaf sheathes of rice (Nōrin 29) seedlings on agar blocks

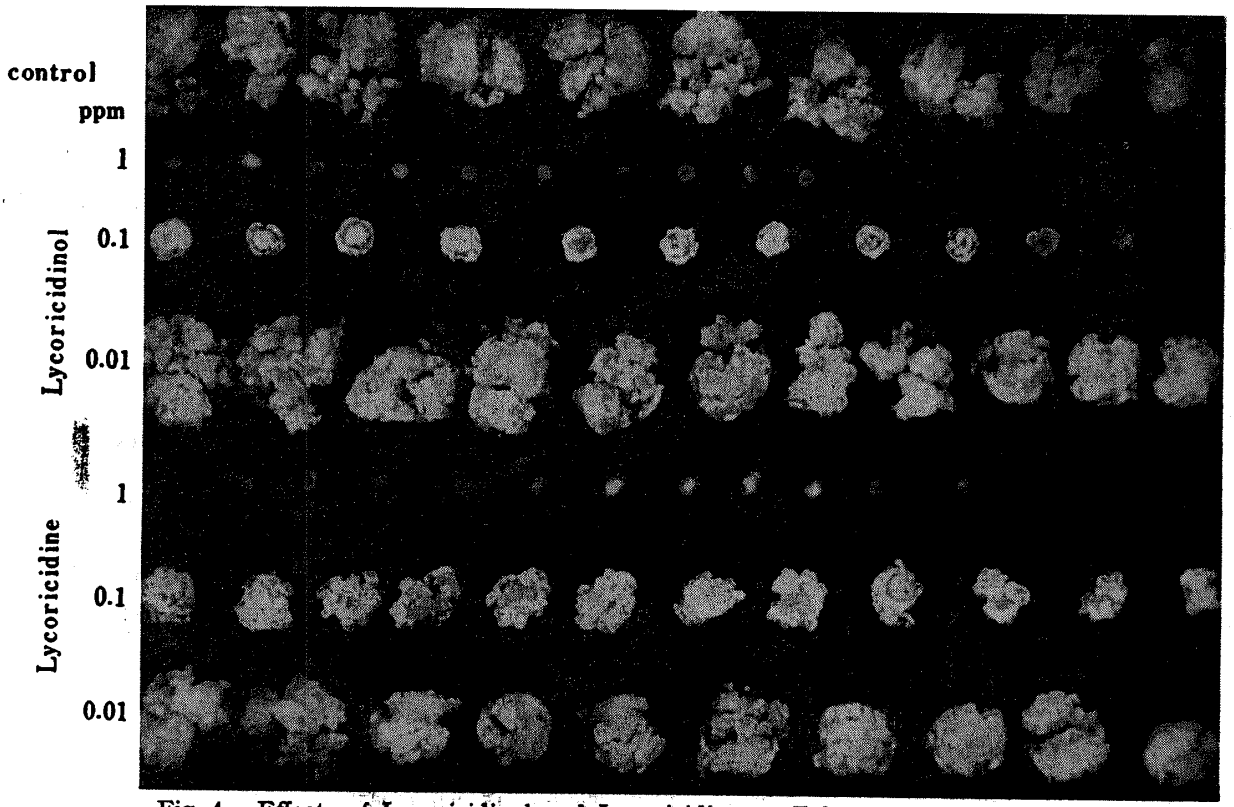


Fig. 4. Effects of Lycoricidinol and Lycoricidine on Tobacco Tissue Culture

lycoricidine must be expressed by the formulae **1** and **2**, respectively. The stereochemical problems are being investigated. Narciclasine, isolated from the fresh bulbs of daffodils, for which Piozzi, *et al.*[5] have recently proposed the structure (**13**), seems to be iden tical with lycoricidinol.

Lycoricidinol and lycoricidine have growth-inhibiting activity on *Avena coleoptile* sections and rice seedling test[1] (Fig. 3). They also have marked inhibitory action on cell division in tobacco tissue culture[1] (Fig. 4), and they have carcinostatic activity[6] (Fig. 5).

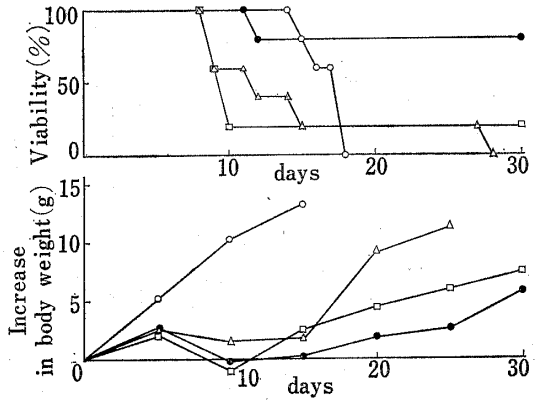


Fig. 5. Antitumor Activity of Lycoricidinol and Lycoricidine using Ehrlich Carcinoma

○ control □ lycoricidinol (100 μg)
● mitomycin C (10 μg) △ lycoricidin (100 μg)

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Gas Chromatography of Principal Hexosamines

Recently, gas chromatographic tequniques have been developed for the detection and quantitative estimation of hexosamines by converting them to acetyl[1], trimethylsilyl[2] and N-acetyl-[3] or N-carboethoxy-trimethylsilyl[4] derivatives, although multiplicity of peaks caused by anomeric isomers or decomposition of the derivatives during gas chromatographic separation are disadvantages of these methods.

We have overcome the difficulties by converting hexosamines to corresponding amino alcohols with sodium borohydride followed by trifluoroacetylation[5] of them with trifluoroacetic anhydride (TFAA) in tetrahydrofuran (THF). The procedure was as follows:

1) H.G. Jones, J.K.N. Jones and M.B. Perry, *Can. J. Chem.*, **40**, 1559 (1962).
2) J. Kärkkäinen, A. Lehtonen and T. Nikkari, *J. Chromatog.*, **20**, 457 (1956).
3) C.C. Sweeley and B. Walker, *Anal. Chem.*, **36**, 1461 (1964).
4) M.D.G. Oates and J. Schrager, *J. Chromatog.*, **28**, 232 (1967).
5) Z. Tamura and T. Imanari, *Chem. Pharm. Bull.* (Tokyo), **15**, 246 (1967).