one hydroxyl attached to each isoquinoline nucleus, and the orientation of these groups was determined as follows: with diazoethane, espinine yielded the *O*-triethyl derivative (Ic), which when reduced with sodium/ammonia afforded (+) N-methyl-6-*O*-ethylisococlaurine (IIIa) [oxalate: mp 210–211° (Kofler), $[\alpha]_D + 103°$ (MeOH)], and (−) N-methyl-*O*,*O*-diethylcoclaurine (IIIb), amorphous, $[\alpha]_D - 64°$ ($CHCl_3$). The NMR-spectra of (IIIa) and (IIIb) are in good agreement with those published by TOMITA et al.[4] for these substances.

From these results, the structure and stereochemistry (Ia) for espinine are established.

Espinidine (Id) appeared to be an *O*-methylespinine from its molecular formula and NMR-spectrum: it showed 3 methoxyls at $\tau$ 6.43 and 6.21 (6 protons), as well as 2 methylimino groups at $\tau$ 7.56 and 7.45. With diazomethane it gave an *O*-dimethyl derivative, $[\alpha]_D + 21°$ ($CHCl_3$), whose NMR-spectrum was identical with that of *O*-trimethylespinine (Ib). The mass spectrum of espinidine showed a weak molecular ion at m/e 610 (0.3%); the rest of the spectrum was practically identical with that of espinine, so that the extra methoxyl must be located at the 4″ position, as in (Id). The structure was confirmed by conversion of espinidine by means of diazoethane into its *O*,*O*-diethyl derivative (Ie) [m.s. $M^+$ 666; m/e 220 (100%) (II, R=Et, R′=Me and vice versa)], which was reduced with sodium/ammonia. The products were identified as (+) N-methyl-6-*O*-ethyl-isococlaurine (IIIa) [oxalate, mp 211–213° (Kofler), $[\alpha]_D + 108°$ (MeOH)] and (−) N-methyl-7-*O*-ethyl-4′-*O*-methylcoclaurine (IIIc), $[\alpha]_D - 38°$ ($CHCl_3$); the NMR data were again in good accord with those published by TOMITA et al. for these bases[4]. Thus espinidine has the structure and stereochemistry represented by (Id).

The structure and configurations of espinine and espinidine suggest that they are formed biogenetically from 1 coclaurine and 1 isococlaurine unit by phenol oxidation, and that they in turn may be further oxidized to lauberine and *O*-methylisothalicberine which occur in the same plant. We are at present studying these possibilities, and work is also under way on the isolation of the 3 remaining phenolic alkaloids from this plant[5,6].

*Zusammenfassung.* Die Isolierung von zwei neuen dauricinartigen Alkaloïden, Espinin and Espinidin, aus *Berberis laurina*, und deren Strukturaufklärung durch Spektroskopie und Degradation werden beschrieben.

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[5] TLC data were obtained on $SiO_2$ G with cyclohexane-chloroform-diethylamine 5:4:1. NMR-spectra were run in $CDCl_3$ with TMS as internal standard. Mass spectra were run on an MS9 instrument.

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## Lentinacin: a New Hypocholesterolemic Substance in *Lentinus edodes*

For the purpose to isolate biologically active substance, we have long been investigating a number of mushrooms. During these studies, we have succeeded in isolating a new hypocholesterolemic substance (designated as lentinacin) from an edible mushroom, 'Shiitake' (*Lentinus edodes*), as pure crystals and confirmed its chemical structure as 2(R),3(R)-dihydroxy-4-(9-adenyl)-butyric acid (I) by spectrometry, degradation study and further total synthesis. KANEDA and TOKUDA[1] also reported the hypocholesterolemic effect of *Lentinus edodes*, the active substance, however, has not been identified.

The caps of dried mushroom, *Lentinus edodes* (1 kg) were homogenized and extracted with 10 l of 80% ethanol. The extract was applied to a column of Amberlite IR-120 (form $H^+$) and the adsorbed substances were eluted with 4% $NH_4OH$. The eluate was evaporated to dryness and the residue was dissolved in water. This solution was passed through a column of Amberlite IR-45 (form $Cl^-$) and eluted with *N* acetic acid. Separation of lentinacin from the concentrated eluate was achieved with a Hitachi preparative amino acid analyzer, model KLA-III, specially equipped with an UV-absorption detector. The column of the analyzer, 3.6 × 150 cm, contained the buffered analytical resin, Amberlite CG-120[2]. The analyzer was operated at 50 °C using a buffer flow rate of 480 ml/h. The effluent volume between 6300 and 7280 ml, having UV-absorption at 260 nm, was collected. This solution was desalted by cation-exchange resin and concentrated.

Lentinacin crystallized from hot water as colourless needles (483 mg), mp 261–263° (dec.); Anal.: C, 42.72; H, 4.58; N, 27.50 and corresponded to the molecular formula $C_9H_{11}O_4N_5$ (mol. wt. = 253 by mass spectrometry of methyl ester). The compound exhibits a characteristic UV-absorption spectrum of 9-substituted adenine ($\lambda_{max}^{N\,HCl} = 259.5$ nm, $\varepsilon = 14{,}179$; $\lambda_{max}^{H_2O} = 261.5$ nm, $\varepsilon = 14{,}508$; $\lambda_{max}^{N\,NaOH} = 262$ nm, $\varepsilon = 14{,}306$).

Sodium salt is readily obtained by treating lentinacin with $NaHCO_3$. Recrystallization from 70% ethanol yielded colourless leaflets, mp 266–268° (dec.); $[\alpha]_D^{20} = +45.5°$ (C = 1 in $H_2O$). Elementary analysis gave values consistent with the hemihydrate. The IR-spectrum of sodium salt is shown in Figure 1. The NMR-spectrum had the following signals: (100 MHz in $D_2O$) singlets at $\delta$ (ppm) 8.04 and 8.03 (2 non-coupled protons), and multiplets at 4.40–4.10 (4 protons).

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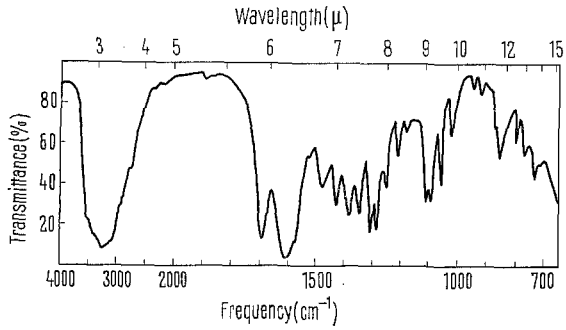


Fig. 1. IR-spectrum of lentinacin Na salt (in KBr).

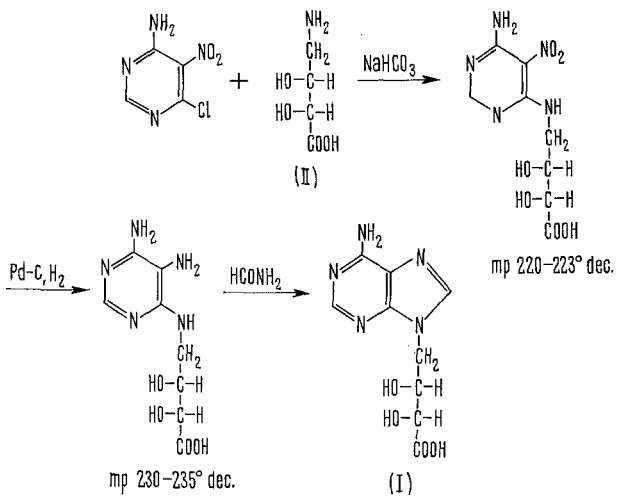


Fig. 2. Synthetic pathway of lentinacin.

Cleavage of lentinacin with 6*N* HCl (110 °C, 72 h) resulted in the liberation of glycine and a new amino acid, 4-amino-2, 3-dihydroxybutyric acid (II). The amino acid (II) was obtained as colourless pillars from water, mp 215–217° (dec.); $[\alpha]_D^{20} = +36.9°$ (C = 0.25 in $H_2O$, calculated from the ORD curve). Anal. ($C_4H_9O_4N$): C, 35.25; H, 6.68; N, 10.10. The structure of II was confirmed by the synthesis through 4-amino-4-deoxy-2, 3-*O*-isopropylidene-D-erythronic acid[3].

The diol linkage in lentinacin was also elucidated by periodate oxidation.

The degradation studies and spectrometric data indicate I for the structure of lentinacin.

Total synthesis of lentinacin was carried out by the procedure illustrated in Figure 2. The physicochemical properties of synthetic lentinacin coincided with those of natural lentinacin. As presented in the Table, synthetic lentinacin showed completely the same marked hypocholesterolemic effect as natural lentinacin in Sprague-Dawley rats. Its acute and chronic toxicities in experimental animals were extremely low.

Effect of lentinacin on serum total cholesterol levels in rats[a]

| Supplement[b] | Serum total cholesterol level[c] | | |
|---|---|---|---|
| | Day 0 mg/100 ml | Day 7 mg/100 ml | Decrease[d] % |
| None | 83 ± 3.2 | 78 ± 3.1 | 6 ± 2.6 |
| 0.005% Lentinacin | | | |
| natural | 83 ± 4.4 | 63 ± 4.4 | 25 ± 1.8 |
| synthetic | 83 ± 6.2 | 62 ± 5.3 | 25 ± 5.3 |
| 0.01% Lentinacin | | | |
| natural | 83 ± 3.7 | 60 ± 2.1 | 28 ± 2.7 |
| synthetic | 83 ± 5.2 | 60 ± 5.0 | 28 ± 2.3 |

[a] Male rats of the Sprague-Dawley strain weighing 140–160 g. [b] Lentinacin was supplemented as sodium salt to a commercial stock diet purchased from Japan CLEA Company. [c] Mean values of 5 rats ± S.E. Total cholesterol was determined by the modified method of ZAK[4]. [d] $\left(1 - \frac{\text{Serum total cholesterol on Day 7}}{\text{Serum total cholesterol on Day 0}}\right) \times 100$.

It is very interesting that this newly found and synthesized adenine derivative, which was originally isolated from edible mushroom, shows high hypocholesterolemic activity. We are now investigating the biosynthetic pathway of lentinacin as well as the mechanism of its hypocholesterolemic action.

*Zusammenfassung.* Aus dem Speisepilz «Shiitake» (*Lentinus edodes*) wurde ein neues Adeninderivat isoliert und Lentinacin genannt. Seine chemische Struktur wurde mit 2(R), 3(R)-Dihydroxy-4-(9-adenyl)-buttersäure durch die vollständige Synthese identifiziert. Lentinacin senkt den Cholesterinspiegel bei Ratten.

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## A New Peptide Coupling Agent – Phosphonitrilic Chloride

Various organic chlorophosphites are employed as coupling agents in peptide chemistry[1]. However, the related inorganic cyclic phosphonitrilic chloride (PNC) has not been evaluated in this aspect, although the chemistry of the compound has been studied for some years[2]. The commercial product contains mostly trimer with some tetramer and a trace of higher oligomers, yet may be fractionated to produce a pure reagent[3].

In a typical experiment, the triethylammonium salt of N-benzyloxycarbonyl-L-phenylalanine (1 mmole) was