Teil die Arbeiten des Karrerschen Instituts auf dem Gebiet der Alkaloidchemie. Dabei werden vor allem die Bedeutung der Strukturaufklärung des Lupinins und die Arbeiten zur Strukturaufklärung von Spartein betont. Anschliessend folgt eine Besprechung der Arbeiten auf dem Gebiet der Kalebassen-Kurarealkaloide, soweit dies in einem solchen Rahmen überhaupt möglich ist. In etwa 60 Publikationen wurde hier ein riesiges Tatsachenmaterial zusammengetragen, und es sind chemische Probleme von ungeheurer Komplexität gelöst worden.

Im zweiten Teil der Arbeit werden einige heute besonders aktuelle Gebiete der Alkaloidforschung besprochen, so zum Beispiel die Chemie der grossringigen Peptidalkaloide, die in zunehmender Zahl in verschiedenen Pflanzenfamilien aufgefunden werden. Es wird ferner darauf hingewiesen, dass mit den heute verfeinerten Isolierungsmethoden Alkaloide in Pflanzenfamilien aufgefunden werden, wo solche früher nie vermutet wurden. Schliesslich werden einige Pflanzenfamilien aufgeführt, deren Bearbeitung wissenschaftlich besonders aussichtsreich erscheint.

SPECIALIA

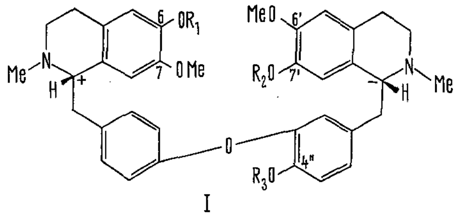
Alkaloids of *Berberis laurina* Billb. II. Two New Phenolic Biscoclaurine Alkaloids

In addition to the alkaloids previously reported[1,2] from the alkali-insoluble fractions of *Berberis laurina* Billb., we have now isolated 2 phenolic alkaloids which we have named espinine (Ia), $C_{36}H_{40}N_2O_6$, and espinidine (Id), $C_{37}H_{42}N_2O_6$.

Espinine, mp 123–125° (Kofler), Rf 0.08, $[\alpha]_D + 25°$ ($CHCl_3$), crystallized from a methanol solution of the alkali-soluble bases, and the mother liquors, submitted to counter-current distribution between benzene and pH 11.9 buffer (120 transfers), afforded 5 distinct bases which gave single spots on TLC. Tubes 1–7 contained espinine, and tubes 64–68 yielded the related amorphous base espinidine, Rf 0.15, $[\alpha]_D + 31°$ ($CHCl_3$).

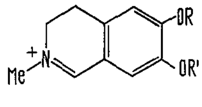
Both bases had practically identical UV-spectra, characteristic of *bis*benzyltetrahydroisoquinoline alkaloids. The NMR-spectrum of espinine showed 2 methylimino groups at τ 7.59 and 7.51, and 2 methoxyls at τ 6.43 and 6.23. The aromatic region showed the presence of 11 protons, including 2 high-field ones at τ 3.98 and 3.75. On treatment with diazomethane, espinine yielded an *O*-trimethyl derivative (Ib), $[\alpha]_D + 14°$ ($CHCl_3$), which shows 5 methoxy groups at 6.39, 6.35, 6.19 and 6.17 (6 protons), as well as 2 methylimino groups at τ 7.50 and 7.46, and the 2 high-field aromatic singlets at τ 3.86 and 3.81.

These data are consistent with a *bis*coclaurine alkaloid of type (I) with a single diaryloxy bridge, and this is confirmed by the m.s. of espinine: the spectrum has comparatively few peaks, the molecular ion (m/e 596) is of very low relative intensity ($< 1\%$) and there are no double-charged ions, as with other bases of this type[3]. The base peak of m/e 192 corresponds to the ion or ions (II, R=H, R'=Me or vice versa), which loses a methyl group to form the ion m/e 177 (26%), as shown by the metastable ion at m/e 163.0. Thus espinine must have one methoxyl and

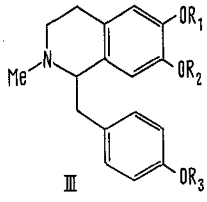


I

	R_1	R_2	R_3
Ia:	H	H	H (Espinine)
Ib:	Me	Me	Me
Ic:	Et	Et	Et
Id:	H	H	Me (Espinidine)
Ie:	Et	Et	Me



II



III

	R_1	R_2	R_3
IIIa:	Et	Me	H
IIIb:	Me	Et	Et
IIIc:	Me	Et	Me

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[1] Part I of this series: M. R. Falco, J. X. de Vries, A. G. de Brovetto, Z. Macció, S. Rebuffo and I. R. C. Bick, Tetrahedron Letters *(1968)*, 1953.

[2] J. Baldas, Q. N. Porter, I. R. C. Bick, G. K. Douglas, M. R. Falco, J. X. de Vries and S. Yu. Yunusov, Tetrahedron Letters *(1968)*, 6315.

[3] D. C. De Jongh, S. R. Shrader and M. R. Cava, J. Am. chem. Soc. *88*, 1052 (1966).

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), $[\alpha]_D + 103^\circ$ (MeOH)], and (−) N-methyl-*O,O*-diethylcoclaurine (IIIb), amorphous, $[\alpha]_D - 64^\circ$ ($CHCl_3$). 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 τ 6.43 and 6.21 (6 protons), as well as 2 methylimino groups at τ 7.56 and 7.45. With diazomethane it gave an *O*-dimethyl derivative, $[\alpha]_D + 21^\circ$ ($CHCl_3$), 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), $[\alpha]_D + 108^\circ$ (MeOH)] and (−) N-methyl-7-*O*-ethyl-4′-*O*-methylcoclaurine (IIIc), $[\alpha]_D - 38^\circ$ ($CHCl_3$); 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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Hobart (Tasmania, Australia), 18 August 1969.

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[4] M. Tomita, T. Shingu, K. Fujitani and H. Furukawa, Chem. pharm. Bull., Tokyo *13*, 921 (1965).

[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.

[6] This work was partly supported by the Comisión de Investigaciones Científicas, Montevideo. We are grateful to Profs. V. Deulofeu and M. J. Vernengo, and to Prof. A. Paladini (University of Buenos Aires) for NMR spectra, and for the use of Craig machine facilities respectively; also to Dr. Q. N. Porter and Mr. J. Baldas (Melbourne University) for determining mass spectra.

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.); $[\alpha]_D^{20} = +45.5^\circ$ (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 δ (ppm) 8.04 and 8.03 (2 non-coupled protons), and multiplets at 4.40–4.10 (4 protons).

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[1] T. Kaneda and S. Tokuda, J. Nutrition *90*, 371 (1966).

[2] S. Moore, D. H. Spackman and W. H. Stein, Analyt. Chem. *30*, 1185 (1958).