Studies on Lignoids in *Lauraceae*. II.[1] Studies on Lignans in the Leaves of *Machilus Japonica* Sieb. et Zucc.

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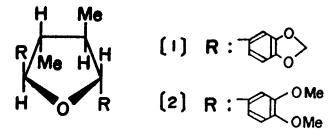
From the leaves of *Machilus japonica* Sieb. et Zucc., four lignans were isolated; they have been found to be (+)-galbacin [**1**], (+)-galbelgin [**2**], (−)-licarin-B [**4**], and a new lignan, machilusin, whose structure can be described as [**3a**] or [**3b**].

The leaves of most species of *Lauraceae* are known to contain the characteristic essential oil, but the systematic studies of the non-volatile components have been few. We planned such systematic studies of the lignans of *Lauraceae* and have already reported the isolation and characterization of several lignans from the leaves of *Cinnamomum Camphora* (Camphor tree).[1]

In this paper, we wish to report some lignans contained in the leaves of *Machilus japonica* Sieb. et Zucc.

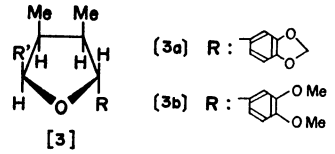
Results

From an acetone extract of fresh leaves of *Machilus japonica* Sieb. et Zucc. (Japanese name: "Hosobatabu"), four lignans were isolated; they have been found to be enantiomers of (−)-galbacin[2] isolated from *Himantandra baccata* Bail (syn. *Galbulimima baccata*), of (−)-galbelgin[3] isolated from *Himantandra belgraveana* F. V. Mull., (−)-licarin-B[4] isolated from *Licaria aritu* Ducke (*Lauraceae*), and a new lignan, machilusin.

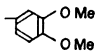


[1] R:

[2] R:

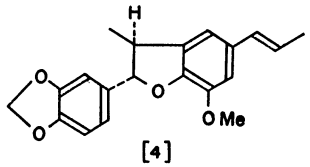


[3]

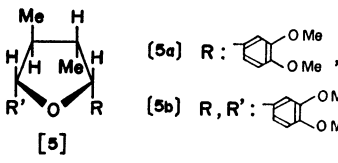
[3a] R: , R': 

[3b] R:

, R': 



[4]



[5]

[5a] R:

, R': 

[5b] R, R':

(+)-Galbacin [*1*]. Oxidation with potassium permanganate affords piperonylic acid. NMR and mass spectra show that this compound is a 2,5-diaryl-3,4-dimethyltetrahydrofuran. Furthermore, the chemical shift of the protons of the two methyl groups in the NMR spectrum indicates that each methyl group is located in *trans* relation to the vicinal aryl group.[5] From this fact, along with the optical activity (dextro), we conclude that this compound is (+)-galbacin [**1**]. The almost identical mp, the absolute value of $[\alpha]_D$, and the mass spectrum[6] confirm this conclusion.

(+)-Galbelgin [*2*]. Oxidation with potassium permanganate affords veratric acid. Mass and NMR spectra and optical activity (dextro) show that this lignan must be the enantiomer of (−)-gabelgin, as in the case of (+)-galbacin. This conclusion is confirmed by the NMR[5] and mass spectra,[6] which are almost identical with those of (−)-galbelgin. The reason why the mp is higher and the absolute value of $[\alpha]_D$ is larger than those reported for (−)-galbelgin is obscure, but we think that the latter might be contaminated with the optically inactive mesoisomer, galgravin, since (−)-galbelgin was isolated along with a very large amount of galgravin from *Himantandra belgrbeana* F. V. Mull.,[3] while we could not find any galgravin in *Machilus japonica* Sieb. et Zucc.

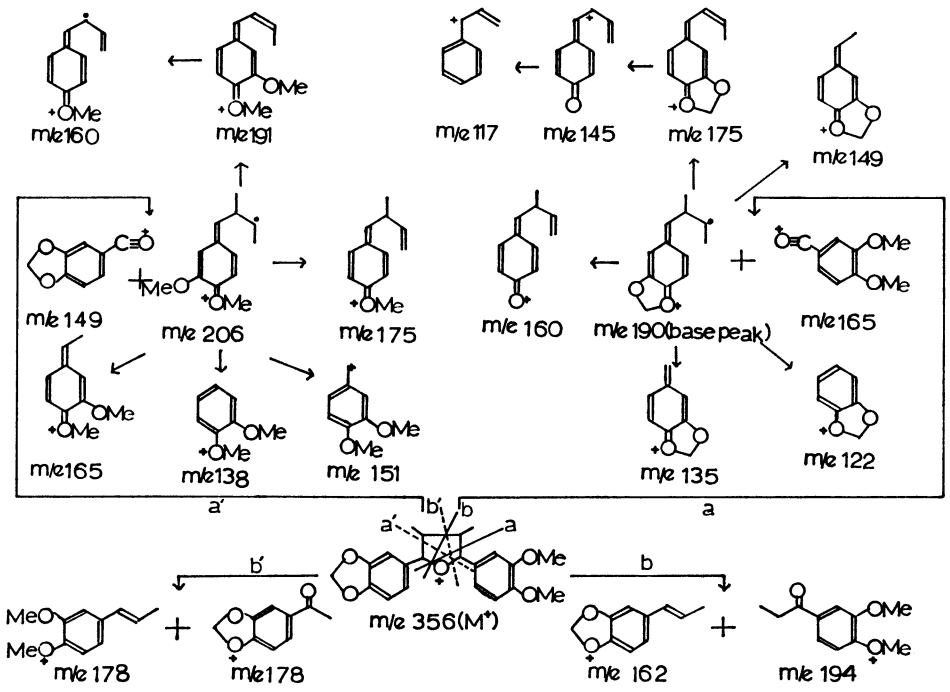
(−)-Machilusin [*3*]. This compound is a new lignan. Oxidation with potassium permanganate affords piperonylic acid and veratric acid (1 : 1). NMR and mass spectra indicate that this compoud is a 2,5-diaryl-3,4-dimethylterahydrofuran.

All of the major peaks of galbacin and galbelgin in the mass spectra have been well established;[6] in the same manner, the fragmentation of machilusin can be explained as represented in Scheme 1.

From the chemical shift of methyl protons in NMR spectrum, it is found that one of the methyl groups is located in *cis* relation and the other in *trans* relation to the vicinal aryl group.[5] Then the relative configuration of this compound can be represented as **3** or **5**.

(+)-Calopeptin[7] and (+)-veraguensin[5] have been reported to have the absolute configurations [**5a**] and [**5b**] respectively. The difference of $[\alpha]_D$ between them is relatively small, and the signals of CH_3CH– in their NMR spectra apear over a relatively wide range, *viz.* from δ 1.5 to 2.5 ppm.[7]

Machilusin is levorotatory and the absolute value of $[\alpha]_D$ differs considerably from the values for calopeptin and veraguensin. The signals of CH_3CH– in the NMR specrum appear in a narrow range, centered at δ 2.4 ppm. These facts indicate that the relative configuration of machilusin must be represented as **3**, *i.e. cis, cis, trans* configuration, not as **5**. In fact, the construction of a molecular model for **3** shows that the two CH_3CH– are so equivalent to each other



Scheme 1.

in the preferential conformation that they would appear in a narrow range in a NMR spectrum.

Then machilusin has either the configuration **3a** or **3b**. **3a** is supposed to be more rational from the viewpoint of the base peak (*m/e* 190) in the mass spectrum, because the bond between C2 and C3 (2,3-bond), bearing two *cis* substituents, may be considered to be cleaved more easily than the 4,5-bond bearing *trans* substituents. A lack of specimen prevented us from performing further studies

Licarin-B [*4*]. The 4th lignan was identified as licarin-B[4] isolated from *Licaria aritu* Ducke, by mp, $[\alpha]_D$ and spectral data.

Experimental

Instruments. $[\alpha]_D$ was measured by the use of Yanako OR-50 (Yanagimoto) and elemental analyses were carried out by CHN Corder MT-2 (Yanagimoto). IR, NMR, and mass spectra were obtained by using IR-400 (Shimadzu), JNM-4H-100 (JEOL), and JMS-01SG-2 (JEOL) respectively.

Isolation of Lignans. Fresh leaves of *Machilus japonica* Sieb. et Zucc., collected at Okitsu, Kochi prefecture, were extracted with acetone; the acetone solution was concentrated and the residue was extracted with hexane. The hexane solution, after concentration, was steam distilled to remove the volatile components and the residue was extracted with hexane. The resulting hexane solution was dried over anhydrous sodium sulfate and, after concentration, was chromatographed on a neutral alumina column (solvent: hexane–ethyl acetate, 95 : 5 to 50 : 50) into 14 fractions.

From the 4th and the 5th fractions, (+)-galbacin, the major lignan, was crystallized in mass, then recrystallized from ethyl acetate (yield: 0.3%).

From the 8th fraction, a white precipitate was filtered and recrystallized from ethyl acetate. The resulting crystals in rod form and the amorphous ones were separated from each other by handpicking. The former were recrystallized from ethyl acetate into pure (+)-gabelgin (yield: 0.005%).

The mother liquid of crude (+)-galbacin, obtained from the 5th fraction, was rechromatographed on a silica gel column (solvent: hexane–ethyl acetate, 95 : 5 to 50 : 50) into 21 fractions. Machilusin was obtained from the 18th fraction by preparative TLC (solvent : hexane–ethyl acetate, 80 : 20) as colorless flat plates (yield: 0.01%).

The 3rd fraction in the first chromatography was rechromatographed on a silica gel column (solvent: hexane–ethyl acetate, 100 : 0 to 60 : 40) into 25 fractions. From the 21st fraction, a pale yellow oil was obtained by means of preparative TLC (solvent: hexane containing 10% ethyl acetate), and this oil was dissolved in a minute amount of hot hexane. On cooling, licarin-B was deposited in white needle-like crystals (a minute amount).

(+)-Galbacin (1). The major lignan, mp 115.5—116 °C, $[\alpha]_D = +117.0°$ (in $CHCl_3$). Found: C, 70.63; H, 5.76%. Calcd for $C_{20}H_{20}O_5$: C, 70.59; H, 5.88%. NMR (δ ppm in $CDCl_3$): 1.03 (6H, d, J=7 Hz; CH_3), 1.75 (2H, m; H3, H4), 4.63 (2H, d, J=9.5 Hz; H2, H5), 5.97 (4H, s; OCH_2O), 6.75—7.0 (6H; aromatic H).

(+)-Galbelgin (2). Mp 142—142.5 °C, $[\alpha]_D = +129.0°$ (in $CHCl_3$). Found: C, 70.98; H, 7.46%. Calcd for $C_{22}H_{28}O_5$: C, 70.91; H, 7.53%. NMR (δ ppm in $CDCl_3$): 1.07 (6H, d, J=6 Hz; CH_3), 1.82 (2H, m; H3, H4), 3.90 (6H, s; CH_3O), 3.94 (6H, s; CH_3O), 4.69 (2H, d, J=9 Hz; H2, H5), 6.80—7.05 (6H; aromatic H). MS: *m/e* 372 (M^+), 206 (base peak), 194, 191, 178, 175, 165, 138.

(−)-Machilusin (3). Mp 121—122.0 °C, $[\alpha]_D = -130.6°$ (in $CHCl_3$). Found: C, 70.81; H, 6.68%. Calcd for $C_{21}H_{24}O_5$: C, 70.79; H, 6.74%. NMR (δ ppm in $CDCl_3$): 0.62 (3H, d, J=7 Hz; CH_3), 1.01 (3H, d, J=6.5 Hz; CH_3), 2.45 (2H, m; H3, H4), 3.90 (6H, s; CH_3O), 4.65 (1H, d, J=9 Hz; H2 or H5), 5.47 (1H, d, J=4 Hz; H2 or H5), 5.95 (2H, s; OCH_2O), 6.80—7.0 (6H; aromatic H). IR (cm^{-1}, KBr): 1603, 1590, 1510, 1500, 1482, 1463, 1458, 1438, 1418, 1380, 1360, 1344, 1313, 1286, 1262, 1240, 1235, 1220, 1190, 1180, 1152, 1140, 1125, 1090, 1060, 1030, 1020, 1005, 995, 970, 960, 940, 925, 895, 860, 850,

810, 798, 780, 770, 750, 725, 720, 705.

(−)-Licarin-B (4). Mp 86.5—88.0 °C, $[\alpha]_D = -45.4°$ (in $CHCl_3$).

Oxidation of Machilusin (3), Galbacin (1), and Galbelgin (2). To a boiling solution of 50 mg of **3** in 10 ml of acetone, 350 mg of a mixture of potassium permanganate and magnesium sulfate (6 : 5) were added little by little over 30 min and the reaction mixture was refluxed for 6 h. Then it was made alkaline with aq. sodium hydroxide and was filtered. The precipitate was washed with hot water, and the combined washing and filtrate were concentrated *in vacuo* to remove acetone and were washed with ether. The aqueous layer was acidified with dil. sulfuric acid and was extracted with ether. The ether solution was dried over anhydrous sodium sulfate and an excess amount of ether solution of diazomethane was added to this solution. The resulting solution, after being concentrated *in vacuo* to remove unreacted diazomethane, was subjected to gas chromatography (SE 30-golay column, at 150 °C). The product was shown to be a mixture of methyl piperonylate and methyl veratrate (1 : 1) by comparing their R_ts with those of the authentic samples.

In the same manner, galbacin and galbelgin gave methyl piperonylate and methyl veratrate respectively.

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