

(+)-NORTRACHELOGENIN, A NEW PHARMACOLOGICALLY ACTIVE LIGNAN FROM *WIKSTROEMIA INDICA*

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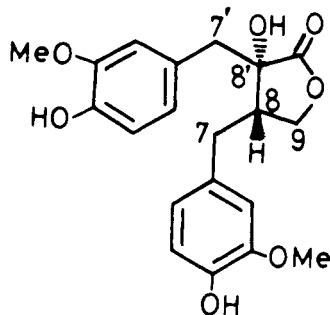
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ABSTRACT.—A new lignan, (+)-nortrachelogenin (I), and a known compound, daphnoretin were isolated from *Wikstroemia indica* C. A. Meyer (Thymelaeaceae). The structure of (+)-nortrachelogenin was established as 8(R), 8'(R)-4,4',8'-trihydroxy-3,3'-dimethoxylignan-olid(9, 9') on the basis of spectroscopic evidence and comparison with its enantiomer, (–)-nortrachelogenin.

(+)-Nortrachelogenin (I) showed effects on the central nervous system producing depression in rabbits.

Wikstroemia indica C. A. Meyer has been used in Formosa as a folk remedy (1) for whooping cough and arthritis. But no other chemical or pharmacological studies of the constituents of this genus have been reported.

Concentrated methanolic extracts of chipped stem which were chromatographed on a silica gel column and on preparative thin layer plates gave crystalline daphnoretin (6-methoxy-7-hydroxy-3,7'-dicoumaryl ether), identified by comparison with published spectral data (2, 3), and resinous (+)-nortrachelogenin (I). Daphnoretin has been isolated previously from *Daphne mezereum* (2) and *Ruta graveolens* (3), though this is the first report of its isolation from the *Wikstroemia* genus.



I

The homogeneous amorphous compound **I** ($[\alpha]_D^{25} +15.4^\circ$ (CHCl_3)) was given a molecular formula, $\text{C}_{20}\text{H}_{22}\text{O}_7$, by the exact mass determination ($+ m/e$ 374.13652). The ir, uv, pmr, and mass spectra data of **I** were identical with those of (–)-nortrachelogenin (**II**)¹ ($[\alpha]_D^{25} -16.8^\circ$ (EtOH)), which was isolated from *Trachospermum asiaticum* Nakai var. (Apocynaceae) by Nishibe, S. et al. (4) (5). Alkaline permanganate oxidation of dimethyl ether of **I** yielded only 3,4-dimethoxy benzoic acid. From these spectroscopic data and the chemical decomposition

¹An authentic sample, (–)-nortrachelogenin (**II**) was furnished by Nishibe, S. (4) (5).

product described above, the planar structure of **I** was established as 4,4',8-trihydroxy-3,3'-dimethoxy-lignan-olid(9,9').

Consequently, the two lignan derivatives are evidently optical isomers, enantiomers, or *cis-trans* isomers. We compared the C^{13} -nmr and CD spectra of **I** with those of **II**, and the following results were obtained. **I** and **II** had identical C^{13} -nmr spectra and, therefore, were probably not *cis-trans* isomers (fig. 1). It

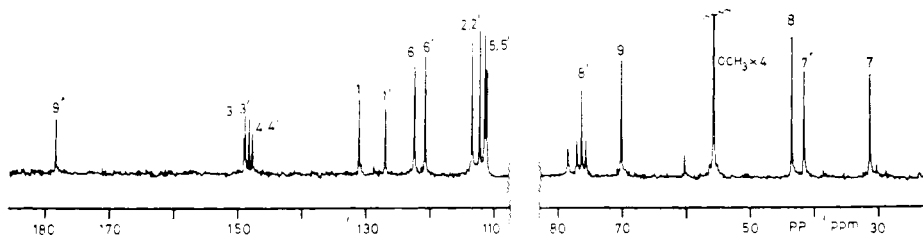
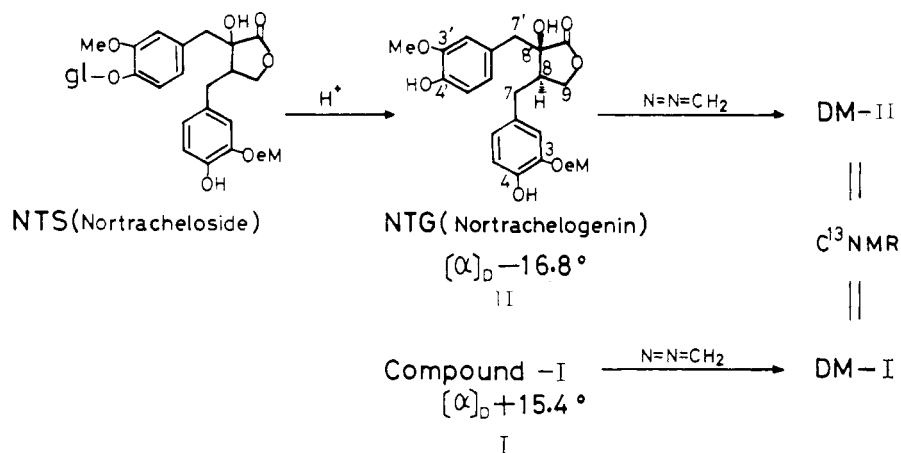


FIGURE 1. $C^{13}NMR$ Spectrum of DM-I or DM-II.

has been shown (6) that usually the C-7, 7', 8, and 8' signals of the *cis* lignan isomer are upfield from the corresponding signals in the *trans* isomer. On the other hand, **I** and **II** in CD measurements showed positive and negative Cotton effect of maximum at 236 $m\mu$ (in MeOH), respectively, as shown in fig. 2.

We ascertained the absolute configuration of the new compound **I**, named (+)-nortrachelogenin, which shows a positive Cotton effect in the CD curve to be 8(R), 8'(R)-4,4',8-trihydroxy-3,3'-dimethoxy-lignan-olid(9,9'), based on the fact that the absolute configuration of the (+)-nortrachelogenin (**II**) has already been elucidated as the 8(S),8'(S)-configuration.

The general pharmacological actions of the methanolic extract from *Wikstroemia indica* and of compounds daphnoretin and **II** isolated from the extract were investigated in mice and rabbits. From the screening tests, it was found that the extract and compound **I** exerted an effect on the central nervous system as seen in table 1. (+)-Nortrachelogenin exhibited also a little activity on convulsion in-

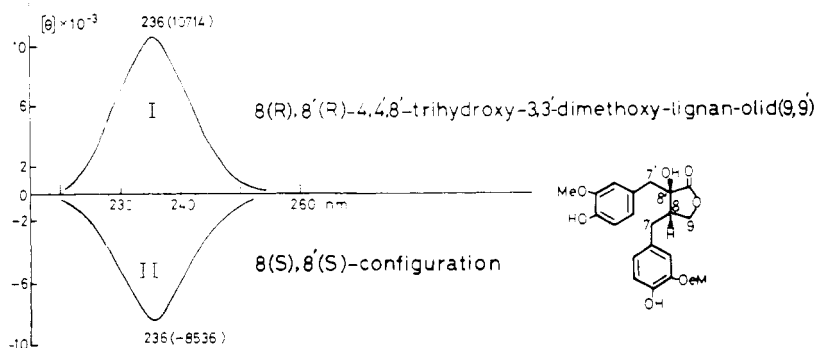


FIGURE 2. CD Curves of (+)-nortrachelogenin (I) and (-)-nortrachelogenin (II).

duced by electroshock, although it did not show tranquilizing and muscle relaxant actions in mice.

TABLE 1. *Effects on central nervous system.*

Drugs	anesthetic enhance (mg/30 mg TPL*)		anti MFM** action	
	Compd. I	MeOH extract	MeOH extract	
Dose.....	60 10	100	100 (mg/kg rabbit)	
Prolong (%).....	823 257	126	25	
Decrease (behavior %).....	—	—	—	
Antielectricity paralysis.....	—	—	—	
LD ₅₀ (mg/kg mouse).....	65	—	—	

*thiopental.

**methamphetamine.

EXPERIMENTAL²

PLANT MATERIAL.—The *Wikstroemia indica* C. A. Meyer (Thymelaeaceae) used in the investigation was collected in Formosa and identified with a herbarium specimen of Taiwan University.

EXTRACTION.—The chipped dry stem of plant material (1 kg) was extracted with MeOH on a heating bath. The methanolic extract was concentrated to a syrup *in vacuo*, mixed with water and filtered to yield a dark brown mealy residue (123 g). The residue was air dried at room temperature and extracted with ether. The ethereal extract, when concentrated, gave a yellow resinous solid (18.5 g).

ISOLATION.—Part (15 g) of this crude resinous solid was chromatographed on a silica gel column with C₆H₆-Me₂CO (5:1). Each fraction was analyzed by tlc (with indicator, ammonium metavanadate 2.0 g in 50% H₂SO₄ soln. 50 ml) in order to group similar fractions. The forerunning fraction, which did not show a spot when sprayed with a solution of ammonium metavanadate, was concentrated to yield pale yellow crystalline daphnoretin (48.5 mg). The next fraction contained a crude substance showing a reddish violet color. The crude resinous substance from the latter fraction, when purified by silica gel preparative tlc chroma-

²Melting points were determined on a W. Buchi TOTTOL melting point apparatus and are uncorrected. Ir spectra were obtained with a Hitachi 215 grating ir spectrophotometer, and uv spectra were recorded with a Hitachi 124 spectrophotometer. Nmr spectra were taken with a Varian NV-21 nmr spectrometer, and chemical shifts were expressed in ppm from TMS as internal reference and coupling constants (J) in Hz. Mass spectra were determined at 75eV on a JEOL-JMS-OGS. in an ion source at 220°. Optical rotations were measured on a Model-DIP-SL of Japan Spectroscopic Co., Ltd. ORD and CD measurements were performed with a spectrometer, Model-ORD/UV-5 Japan Spectroscopic Co., Ltd. Tokyo.

tography with benzene-ethanol (15:1) as a developer, yielded a pale yellow resinous compound (100 mg, $[\alpha]_D +15.4^\circ$ (CHCl₃)).

DAPHNORETIN.—Crystalline daphnoretin, on repeated silica gel column chromatography with acetone-chloroform (1:30), gave 40 mg of pale yellow needles mp. 255°, ν max (KBr): 3250 (phenolic OH), 1730~1710 ($-\text{C}=\text{O}$), 1620, 1570 ($-\text{C}=\text{C}-$) and 1270 cm^{-1} ($=\text{C}-\text{O}-$); ν max (MeOH): 228 (1.18), 265 (0.86), 325 (1.28) and 343 (1.31) nm (log ϵ) (an absorption band of 325 nm indicated a bathochromic shift by addition of alkali); ms m/e M⁺ 352.0582 (352.0582) for C₁₅H₁₂O₇ (100%), 179 (M-CO, -C₅H₅O₂) (47%), 145 (M-CO, -C₅H₅O₂) (34%); pmr (D₂O-DMSO), δ 6.37 (d, 1H, H-3 α -pyrone), 8.01 (d, 1H, H-4 α -pyrone) $J_{3,4}=9.0$ Hz, 6.90 (s, 1H, H-4' of α -pyrone), 7.10 (d, 1H, H-6 of benzene), 7.71 (d, 1H, H-5 of benzene) $J_{5,6}=8.5$ Hz, 7.17 (s, 1H, H-8' of benzene), 7.22 (s, 1H, H-8 of benzene), 7.85 (s, 1H, H-5' of benzene ring), 3.87 (s, 3H, -OCH₃), 3.8 (broad, 1H, OH disappearing with D₂O). Finally, these spectral data were in accord with those published (2) (3).

(+)-NORTRACHELOGENIN (I).—I (100 mg) gave one spot on silica gel tlc with several solvent systems [i.e. benzene-ethanol (15:1), isopropyl ethyl ether-ethanol (15:2), isopropyl ether-ethanol-benzene (15:2:2)]. It was a glassy pale yellow resin. It gave the following physical data $[\alpha]_D +15.4$ ($c=0.52$ in CHCl₃), ν max (CHCl₃): 3450 (OH), 1770 (γ -lactone), 1610, 1598 and 1518 cm^{-1} (aromatic C=C); ν max (MeOH): 230 (2.05), 281.5 (1.35), bathochromic shift 249 (2.65) and 298 (1.75) nm (log ϵ); high resolution ms m/e M⁺ 374.136552 (374.136556 for C₂₀H₂₂O₇), 219 (M-C₅H₅O₂) and 137 (3-methoxy-4-hydroxy-benzyl cation) (base peak); pmr (CDCl₃): 6.55~6.9 (m, 6H, Ar-H₆), 5.65 (broad 3H, 3 x OH), 4.0 (d, 2H, H-9, -O-CH₂-), 3.82 (d, 6H, 2 x OCH₃), 3.0 (AB-q, 2H, H-7', Ar-CH₂-) and 2.4~2.95 (m, 3H, H-7, Ar-CH₂- and 1H, H-8', -CH-). An assignment of the H-7' signals (δ , 3.0, AB-q, 2H of Ar-CH₂-) was suggested by the fact that the proton signals were unchanged when irradiated at the methine proton (H-8 of γ -lactone ring).

(+)-DIMETHYLNORTRACHELOGENIN [(+)-DMNTG].—I (50 mg) in methanol was methylated with diazomethane, and the crude product was recrystallized from methanol to give 40 mg of dimethyl ether, needles, mp. 96°. The (+)-DMNTG gave C¹³-nmr (CHCl₃): δ 31.5 (t, C-7), 4.19 (t, C-7'), 43.7 (d, C-8), 55.9 (q, 4 x OCH₃), 70.3 (t, C-9), 76.5 (s, C-8), 111.3 (d, C-5'), 111.6 (d, C-5), 112.4 (d, C-2'), 113.6 (d, C-2), 120.9 (d, C-6'), 122.6 (d, C-6), 127.1 (d, C-1'), 131.2 (d, C-1), 147.9 (s, C-4' or C-4) and 149.1 (s, C-4 or C-4'). The C¹³-nmr of (+)-dimethylnortrachelogenin (dimethyl ether of I) was identical to that of (-)-dimethylnortrachelogenin.

ALKALINE PERMANGANATE OXIDATION OF (+)-DIMETHYLNORTRACHELOGENIN [(+)-DMNTG].—Oxidation of dimethyl-I (20 mg) with 1N NaOH (10 ml) and 2% KMnO₄ for 1.5 hr. at 30° gave 7 mg of 3,4-dimethoxy benzoic acid which was identified by comparison with an authentic sample.

ORD AND CD MEASUREMENTS OF (+)-NORTRACHELOGENIN (I) AND (-)-NORTRACHELOGENIN (II).—The ord of I gave ($C=0.0262$, CHCl₃) $[\theta]^{27}$ ($m\mu$): +162° (500), +366° (400, trough), +569° (350, trough), +853° (310, trough) ($C=0.000197$, MeOH) $[\theta]^{27}$ ($m\mu$): +508° (300, trough), +1015° (280, trough), +1523° (260, trough), +2538° (250, trough), +3553° (240, peak), +2538° (235, trough), +1523° (230, trough), CD max $[\theta]_{236}^{30} +10714$ ($C=0.000197$, MeOH).

The ord of II gave ($C=0.0251$ CHCl₃) $[\theta]^{27}$ ($m\mu$): -159° (500), -318° (400, trough), -478° (350, trough), -716° (310, trough), CD max $[\theta]_{236}^{30} -8536$ ($C=0.000201$, MeOH).

PHARMACOLOGICAL ACTIVITY OF (+)-NORTRACHELOGENIN (I).—The methanolic extract from *Wikstroemia indica* and I were tested for effect on the nervous system employing the usual procedure (7). The results obtained from the tests are given in table 1.

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