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ABOUT NATIVE COMPONENTS OF EXTRACTS FROM *Maackia amurensis* WOOD

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A new dimeric stilbene, the name for which is suggested as maackin A, is isolated from the wood of Maackia amurensis. *The structure of maackin A is determined by NMR, UV, and IR spectral methods. The* cis-*isomers that are observed during fractionation of the extracts are not native components of the wood but are formed from the* trans-*dimers under certain separation conditions.*

We have previously reported the chemical components in alcohol extracts of the heartwood of *Maackia amurensis*. In addition to isoflavones and monomeric stilbenes [1], their addition product, isoflavonostilbene [2], dimeric stilbenes [3], and stilbenolignan [4] were also isolated. Multiple chromatography on silica-gel columns in various solvent systems was used.

Separation of the fraction with dimeric stilbenes enabled the identification of three compounds: scirpusin *A*, scirpusin *B*, and maackin. However, difficulties arising during further investigations of the polar fractions of the extracts prompted us to seek new approaches for studying their composition. Reverse-phase chromatography on C-18 silica gel in aqueous methanol acidified with formic acid was rather effective for preparative separation of the dimeric stilbenes that we obtained earlier and for the detection of a new compound, a structural isomer of scirpusin *B*, for which we propose the name maackin *A* (**1**). The separation procedure also revealed and isolated *cis*-isomers of these dimers according to UV and PMR spectral data.

The question of whether the *cis*-derivatives are native remained unanswered because the methods available to us for analyzing the initial extract were not able unambiguously to establish their presence or absence.

The goal of the present investigation was to determine if the dimeric *cis*-stilbenes that are observed in the alcohol extracts of *Maackia amurensis* heartwood are native. The structure of a new dimeric stilbene was also determined.

The presence of *cis*-stilbenes in plants was first reported in 1962. However, the only reliable source of *cis*-resveratrol was for a long time *Eucalyptus wandoo* [5]. The question of whether *cis*-stilbenes are present in various plant materials continues to be debated today. Natural substrates are suggested to occur in nature, as a rule, as the more stable *trans*-isomers. Reports describing the simultaneous presence in plants of monomeric *cis*- and *trans*-stilbenes began to appear in 1976. Thus, *cis*- and *trans*-resveratrol was observed in peanuts [6]. Later, polyhydroxylated *cis*- and *trans*-stilbenes were isolated from the stems of *Phoenix dactilifera* [7]. Rhubarb root (*Rhei rhizoma* Polygonaceae) yielded five monomeric *cis*-stilbenes in 1984 [8]. The *cis*- and *trans*-isomers of 3,4′,5-trimethoxystilbene were found in *Ferula latipinna* [9].

A series of monomeric *cis*-stilbenes named combretastatins was isolated from woody African plants of the Combretaceae family. The first representative is combretastatin *A*-1 from *Combretum caffrum* [10], which is especially interesting because it actively inhibits the growth of P-388 lymphatic leukemia tumor cells. Combretastatins *A*-2 [11] and *A*-4 [12] exhibit the same properties. The isomeric oligostilbenes *trans*- and *cis*-vitisin *A* were found in *Vitis coignetiae* [13]. However, they were separated later using HPLC [14]. The detection and isolation of pure dimeric *cis*-stilbenes from *Maackia* abstracts, which may possess antitumor activity, is definitely of scientific and practical interest.

Thus, keeping in mind the literature, the presence of *cis*-stilbenes in *Maackia* wood extracts must be considered possible. It must also be determined if the *cis*-dimers that we observed are artifacts that formed during the extraction or during subsequent separation of the extracts and preparation of the pure compounds. For this the pure dimeric *cis*-stilbenes had to be obtained in

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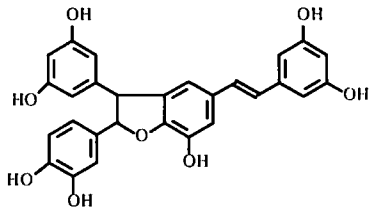
order to use them as model compounds for analyzing the whole extract.



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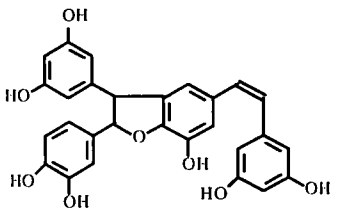

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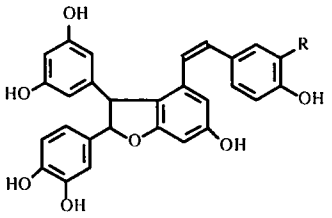

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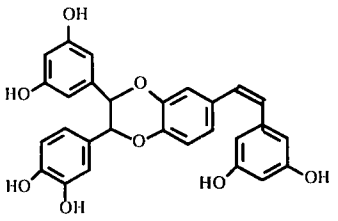

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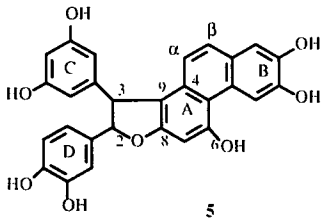

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Samples of dimeric *cis*-stilbenes were obtained by isomerizing the *trans*-derivatives in acidified aqueous alcohol on a reverse-phase C-18 silica-gel chromatography column with a UV detector (254 nm).

Maackin *A* (**1**) and the *cis*-isomers of maakin *A* (**1a**), scirpusins *A* (**2**), *B* (**3**), and maackin (**4**) were obtained first and characterized by UV, IR, CD, and NMR spectra.

PMR spectra of maackin *A* differ from those of scirpusins *A* and *B* and maackin in that the protons on the second and third C atoms have large spin—spin coupling constants (SSCC). The structure of the dihydrobenzofuran moiety was proved by ^{13}C spectra. The minimal difference between the calculated and measured chemical shifts was observed for this structure.

The PMR spectra of the *cis*-isomers contained two doublets with SSCC 12 HZ belonging to the olefinic protons at stronger field than for the *trans*-isomers (SSCC 16 Hz). Furthermore, the *trans*- and *cis*-isomers have markedly different chemical shifts and SSCC for the protons on the second and third C atoms. For example, the PMR spectrum of scirpusin *B* has doublets at 5.36 and 4.46 ppm with SSCC 4.6 Hz whereas the corresponding doublets for *cis*-scirpusin *B* occur at 5.24 and 4.07 ppm with SSCC 5.1 Hz (Table 1).

The stilbene isomers are readily distinguished by the UV spectra. Dimeric *trans*-stilbenes exhibit maxima at 280 and 320 nm. The corresponding *cis*-isomers have only one maximum at 280 nm.

Cassigarol *D* (**5**), which was isolated previously from *Cassia garrettiana* Leguminosae [15], was detected in small quantities (up to 3% of the total content) during the reverse-phase HPLC separation (UV detection) of the fraction containing the dimeric stilbenes. Cassigarol *D* was characterized by PMR spectra. However, it could not be isolated pure in large quantities. It was most likely formed in acidic medium from *trans*-scirpusin *B* through its *cis*-isomer (see below).

Samples of the *cis*-isomers of dimeric stilbenes were used as model compounds for HPLC analysis of the total alcohol extract from *Maackia amurensis* heartwood. The *cis*-isomer was not found in the extracts. Adding the model *cis*-scirpusin *A* to the fraction containing the dimeric stilbenes produced a peak with a retention time of 4.05 min (the retention time of *trans*-scirpusin *A* is 5.28 min).

Two narrow fractions of *trans*-stilbenes were obtained by separating the total fraction of dimeric stilbenes on a silica-gel column using a benzene—acetone gradient increasing from 1 to 30% acetone. The first fraction contained scirpusin *A* and maackin (70 and 23%, respectively, according to PMR spectra). The second contained scirpusin *B* and maackin *A*, together totaling about 88%. Doublets at 4.46 and 5.38 ppm (H-3 and H-2, respectively) for *trans*-scirpusin *A* and a singlet at 4.86 ppm

TABLE 1.^{1}H NMR Data for Dimeric Stilbenes (TMS, Acetone-D_6, δ, SSCC, Hz)

H-atom	Compound								
	1		**2**		**3**		**4**		**5**
	trans	*cis*	*trans*	*cis*	*trans*	*cis*	*trans*	*cis*	-
2	5.39 d:7.5	5.30 d:8.5	5.38 d:5.2	5.25 d:5.2	5.36 d:4.6	5.24 d:5.1	4.86 s	4.80 s	5.41 d:5.0
3	4.45 dd:7.5 0.7	4.40 d:8.5	4.46 d:5.2	4.04 d:5.2	4.46 d:4.6	4.07 d:5.1	4.86 s	4.80 s	4.66 d:5.0
4	6.77 d:2.1	6.46 d:2.1	-	-	-	-	-	-	-
5	-		6.34 d:2.1	6.30 s	6.33 d:2.1	6.29 d:2.1	7.18 d:2.1	6.91 d:1.8	-
6	7.04 d:2.1	6.83 d:2.1	-	-	-	-	-	-	-
7	-	-	6.73 d:2.1	6.30 s	6.72 d:2.1	6.33 d:2.1	7.12 dd:2.1 8.5	6.86 d:1.8 8.5	7.20 s
8	-	-	-	-	-	-	6.95 d:8.5	6.82 d:8.5	-
α	6.86 d:16.2	6.30 d:12.2	6.73 d:16.2	6.01 d:12.2	6.69 d:16.2	5.99 d:12.2	6.98 d:16.2	6.42 d:12.5	7.08 d:8.9
β	6.99 d:16.2	6.41 d:12.2	6.92 d:16.2	6.24 d:12.2	6.86 d:16.2	6.19 d:12.2	7.02 d:16.2	6.45 d:12.5	7.79 d:8.9
B2	6.54 d:2.1	6.34 d:2.1	6.75 d:8.0	6.66 d:8.0	6.72 s	6.73 d:2.1	6.57 d:2.1	6.32 d:2.0	9.26 s
3	-	-	7.19 d:8.0	7.02 d:8.0	-	-	-	-	-
4	6.26 t:2.1	6.22 t:2.1	-	-	-	-	6.29 t:2.1	6.24 t:2.0	-
5	-	-	7.19 d:8.0	7.02 d:8.0	6.72 S	6.66 d:8.2	-	-	6.82 s
6	6.54 d:3.1	6.34 d:2.1 -	6.75 d:8.0	6.66 d:8.0	6.72 s	6.66 dd:8.2 2.1	6.57 d:2.1	6.32 d:2.0	-
C2	6.20 d:2.1	6.14 d:2.1	6.25 s	6.07 d:2.2	6.24 s	6.07 d:2.1	6.24 d:2.1	6.21 br.s.	6.16 d:2.1
4	6.28 t:2.1	6.23 t:2.1	6.25 s	6.21 t:2.2	6.24 s	6.23 t:2.1	6.26 t:2.1	6.24 br.s.	6.23 t:2.1
6	6.20 d:2.1	6.14 d:2.1	6.25 s	6.07 d:2.2	6.24 s	6.07 d:2.1	6.24 d:2.1	6.21 br.s.	6.16 d:2.1
D2	6.90 d:2.1	6.89 d:2.1	6.84 d:2.1	6.77 d:2.2	6.87 d:2.1	6.77 d:2.1	6.80 d:2.1	6.74 br.s.	6.85 d:2.1
5	6.83 d:8.2	6.82 d:8.2	6.82 d:.8.2	6.79 d:8.2	6.81 d:8.0	6.80 d:8.2	6.72 d:8.2	6.69 d:8.2	6.81 d:8.2
6	6.75 dd:8.2 2.1	6.73 dd:8.2 2.1	6.72 dd:8.2 2.1	6.60 dd:8.2 2.2	6.71 dd:8.0 2.1	6.62 dd:8.2 2.1	6.59 dd:8.2 2.1	6.54 br.d:2.2	6.72 dd:8.2 2.1

(2H, H-2 and H-3) for *trans*-maackin were observed in the PMR spectrum. The PMR spectrum of *trans*-scirpusin *B* contains a doublet at 4.46 and 5.36 ppm with SSCC 4.6 Hz. A doublet of doublets at 4.45 ppm with SSCC 7.5 and 0.7 Hz and a doublet at 5.39 ppm with SSCC 7.5 Hz appear in the spectrum of *trans*-maackin *A*.

Cassigarol *D* was not detected in these fractions. However, it did form (~3%) during reverse-phase chromatography using acidified aqueous alcohol with UV detection during separation of the second fraction, which contained scirpusin *B*. Direct

irradiation by UV light (low-pressure mercury lamp) for 6 h of a solution of scirpusin *B* produced *cis*-scirpusin *B* and cassigarol *D*, the contents of which were 33 and 30%, respectively, according to PMR spectra. Such irradiation of the three other stilbenes did not produce cassigarol *D*.

Thus, it should be noted that dimeric *cis*-stilbenes and cassigarol *D* are not native components of the extract of *Maackia amurensis* and are not formed during the extraction or evaporation of the extracts or during silica-gel column separation in neutral solvents in ordinary daylight.

The *cis*-isomers are formed by isomerization of the native *trans*-stilbenes of the wood extract under certain separation conditions, in our instance, on a C-18 silica-gel column in acidified aqueous alcohol with a flow-through UV detector. Resveratrol and piceatannol, which are components of *Maackia amurensis* extracts, isomerize under these same conditions but to a lesser degree than dimeric stilbenes, which have bulky and heavy substituents on the double bond. It is necessary to clarify at what stage of the separation the isomerization occurs.

Recently the observation of *cis*- and *trans*-resveratrol in red wines has caused great excitement. Several groups of analytical chemists have developed quantitative chromatographic methods for determining these components [16]. Many reproducible experiments confirmed the presence of native *cis*- and *trans*-isomers. Therefore, analytical HPLC had little effect on the ratio of resveratrol isomers. HPLC applied to the analysis of the total alcohol extract of *Maackia* could also clearly distinguish native *trans*-isomers and model *cis*-isomers.

Conditions are different for preparative separation of dimeric stilbenes. The process lasts from 3 to 6 h. The fraction containing *trans*-scirpusin *A* and *trans*-maackin was separated on a previously used (see above) silica-gel column in acidic aqueous alcohol without UV detection. According to PMR, scirpusin *A* was isomerized into the *cis*-isomer by 17%; maackin, by 9%. It is important that the isomerization of this same fraction in acidic aqueous alcohol with heating to 60°C for 6 h gave only 1.5% of the *cis*-isomers according to PMR spectra. Therefore, not only the acidic medium but also the sorbent plays an important role in the isomerization. The conversion of *trans*-stilbenes into the *cis*-isomers occurs on the sorbent surface.

Brief exposure of stilbenes in the flow cell to the UV detector does not cause noticeable isomerization of monomeric and dimeric stilbenes.

EXPERIMENTAL

A glass column with KSK silica gel was used for preparative column chromatography. The mobile phases were benzene:acetone and $CHCl_3$:acetone with a gradient increasing from 1 to 30% acetone. Silufol plates were used for TLC.

Reverse-phase chromatography was performed on C-18 silica gel using CH_3OH:water (70:30) with added formic acid (0.05 ml) per liter. A Uvicord SLKB UV-detector (254 nm) was used.

HPLC was carried out on a GSP 100 (Czech Republic) instrument with a Separon SGX C18.5 μm (15×0.33 cm) column. The mobile phase was CH_3CN with 2% aqueous acetic acid (20:50). The flow rate was 1 ml/min. Wavelength 254 nm was monitored.

The isolated pure compounds were identified using physicochemical methods. NMR spectra were recorded on a Bruker WM-250 instrument at working frequencies 250 (^1H) and 62.9 MHz (^{13}C) (δ, ppm, 0 - TMS). Mass spectra were obtained on a LKB-9000 S mass spectrometer by direct insertion into the ion source at 15 and 70 eV. UV spectra were recorded on a Spekord M40 spectrophotometer in CH_3OH; IR spectra, on a Specord M82 in dioxane-D_8.

Maackin *A* (1), olive-colored powder, $C_{28}H_{22}O_8$, *m/z*: 244, 137; UV [λ_{max}, CH_3OH, nm (lg ε)]: 204 (4.84), 233 sh, 288 sh, 313 sh, 327 (4.24); IR (dioxane-D_8, ν, cm^{-1}): 3330 (OH), 1608, 1509 (C=C); CD (*s* 4.0, CH_3OH): $[\theta]_{260} = +920$ (br); ^{13}C NMR (acetone-D_6, δ, ppm): 58.5 (C-3), 94.8 (C-2), 115.5 (C-4), 132.7 (C-5), 115.0 (C-6), 141.9 (C-7), 145.2 (C-8), 132.4 (C-9); ring *B*: 140.6 (C-1), 105.7 (C-2), 159.2 (C-3), 102.4 (C-4), 159.2 (C-5), 105.7 (C-6); ring *C*: 148.0 (C-1), 107.3 (C-2), 159.4 (C-3), 102.7 (C-4), 159.4 (C-5), 107.3 (C-6); ring *D*: 133.5 (C-1), 114.0 (C-2), 145.9 (C-3), 145.8 (C-4), 115.9 (C-5), 118.8 (C-6), 127.2 (C-α), 129.3 (C-β).

Maackin *A-cis* (1a), light olive-colored powder, $C_{28}H_{22}O_8$; *m/z*: 244, 226, 137; UV [λ_{max}, CH_3OH, nm (lg ε)]: 204 (4.93), 233 sh, 286 (4.19); IR (dioxane-D_8, ν, cm^{-1}): 3315 (OH), 1608, 1508 (C=C); CD (*c* 4.0, CH_3OH): $[\theta]_{248} = +1300$.

Scirpusin *A-cis* (2), light olive-colored powder, $C_{28}H_{22}O_7$; *m/z*: 244, 226, 137; UV [λ_{max}, CH_3OH, nm (lg ε)]: 204 (4.64), 227 sh, 284 (3.94); IR (dioxane-D_8, ν, cm^{-1}): 3325 (OH), 1609, 1588 sh, 1516 (C=C); CD (*c* 4.0, CH_3OH): $[\theta]_{220} = +175$, $[\theta]_{260} = -1067$.

Scirpusin *B-cis* (3), light olive-colored powder, $C_{28}H_{22}O_8$; *m/z*: 244, 137; UV [λ_{max}, CH_3OH, nm (lg ε)]: 204 (4.92), 233 sh, 286 (3.90); IR (dioxane-D_8, ν, cm^{-1}): 3325 (OH), 1609, 1588, 1516 (C=C); CD (*c* 4.0, CH_3OH): $[\theta]_{260}$ = -1047.

Maackin-*cis* (4), olive-colored powder, $C_{28}H_{22}O_8$; *m/z*: 244, 137; UV [λ_{max}, CH_3OH, nm (lg ε)]: 204 (4.93), 227 (4.29), 278 (3.59); IR (dioxane-D_8, ν, cm^{-1}): 3320 (OH), 1606, 2514 (C=C); CD (*c* 4.0, CH_3OH): $[\theta]_{220}$ = +74, $[\theta]_{250}$ = -831.

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