



Flavonoids from *Lonchocarpus latifolius* roots

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Abstract

From the petrol extract of *Lonchocarpus latifolius* roots, 10 flavonoids were isolated. These included: 3,5-dimethoxy-2'',2''-dimethylpyrano-(5'',6'':8,7)-flavone, 3-methoxy-(2'',3'':7,8)-furanoflavanone, 3',4'-methylenedioxy-(2'',3'':7,8)-furanoflavanone, and (2,3-*trans*-3,4-*trans*)-3,4-dimethoxy-(2'',3'':7,8)-furanoflavan, as well as the previously known *karanjachromene*, *karanjin*, *lanceolatin B*, *pongachromene*, *pongaglabrone* and *ponganpin*. Only nine flavonoids could be quantified through HPLC analysis. © 2000 Published by Elsevier Science Ltd. All rights reserved.

Keywords: *Lonchocarpus latifolius*; Leguminosae; Roots; Flavone; Flavane; Flavanone

1. Introduction

Of the approx. 100 *Lonchocarpus* species in existence, 24 are native to Brazil (Magalhães et al., 1996). The literature contains reports on the chemistry of thirty-four species, all of which have furnished flavonoids. Of these *L. araripensis* (Do Nascimento and Mors, 1981), *L. nitidus* (Menichini et al., 1982; Gomes et al., 1981), *L. obtusus* (Gomes et al., 1981; Do Nascimento et al., 1976) and *L. sericeus* (Gomes et al., 1981; Mahmoud and Waterman, 1986; Delle Monache et al., 1977) are the only Brazilian species previously investigated by other research groups. *L. nitidus* (Menichini et al., 1982; Gomes et al., 1981) also belongs to the *Densiflori* section of the *Lonchocarpus* subgenus *Lonchocarpus* as does *L. latifolius*, and has furnished chalcones and flavanones. Thus, as part of a chemical study of previously uninvestigated Brazilian *Lonchocarpus* species occurring in Brazil (Magalhães et al., 1996, 1997), we now report the isolation and characterization of ten flavonoids (**1–10**) from the roots of *L. latifolius* which also furnished two dibenzoylmethane derivatives (**11** and **12**) described earlier (Magalhães et al., 1997).

2. Results and discussion

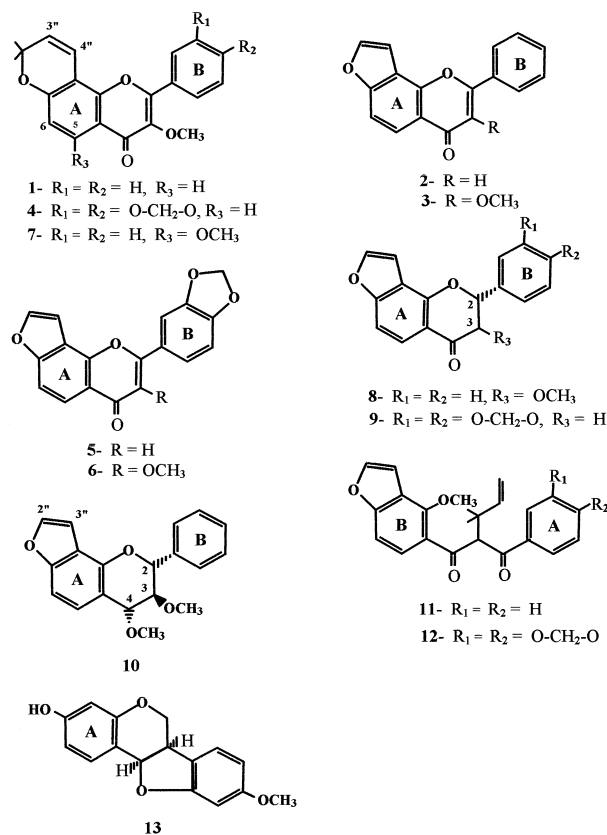
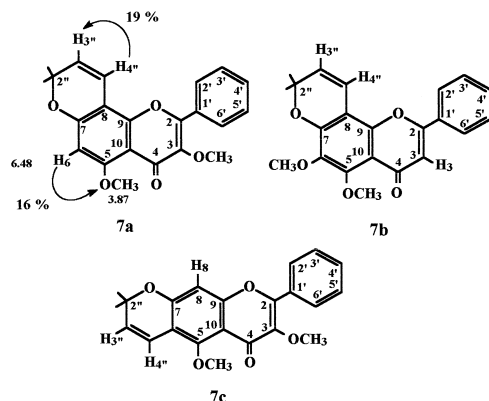
The petrol extract of *Lonchocarpus latifolius* roots on successive chromatographic analysis (column, TLC and preparative TLC) led to flavonoids **1–12** (Fig. 1). *Karanjachromene* **1** (Garcez et al., 1988), *karanjin* **2** (Lyra et al., 1979; Talapatra et al., 1982; Tanaka et al., 1992), *lanceolatin B* **3** (Lyra et al., 1979; Talapatra et al., 1982; Tanaka et al., 1992), *pongachromene* **4** (Garcez et al., 1979; Pelter et al., 1981), *pongaglabrone* **5** (Talapatra et al., 1982; Tanaka et al., 1992; Pelter et al., 1981) and *ponganpine* **6** (Lyra et al., 1979; Tanaka et al., 1992) were characterized by comparing their spectral data with literature values.

Compound **7** was obtained in very small amounts as a yellowish oil. Its ¹H NMR spectrum (see Section 3) was very similar to that of *karanjachromene* (**1**), which also displayed characteristic absorptions for an unsubstituted B ring and a 2,2-dimethylpyran group. However, instead of H-5 and H-6 doublets, there was a singlet at δ 6.48 (1H, *s*) with signals at δ 3.87 (3H, *s*) and δ 3.88 (3H, *s*) indicating the presence of two methoxyl groups. From the three possible structures **7a–c**, structure **7a** was selected based on analysis of the NOE data shown in Fig. 2.

Compound **8** was obtained as a colourless amorphous powder. Its ¹H NMR spectrum (Table 1) was very similar to that of *karanjin* (**2**), except for the presence of

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Fig. 1. Flavonoids isolated from *Lonchocarpus latifolius* roots.Fig. 2. Structural alternatives for compound 7. Only **7a** is compatible with the NOE differential spectrum.

two doublets at δ 5.50 (1H, *d*, $J = 10$ Hz) and δ 4.20 (1H, *d*, $J = 10$ Hz), respectively, typical of H-2 and H-3 absorptions of a flavanone derivative. The diamagnetic shift of one of the methoxyl groups (δ 3.42) was in agreement with that expected for a flavanone 3-methyl ether. HR-EIMS indicated ($M^+ = 294.0894$ suggesting $C_{18}H_{14}O_4$), with mass spectral fragments **8a** [$m/z = 160$] and **8b** [$m/z = 134$], resulting from RDA cleavage of the C ring, and the fragment **8c** [$m/z = 91$] (Fig. 3). All are diagnostic of flavanone derivatives (Harborne et al., 1975) (Table 2).

Compound **9** was obtained as a viscous yellowish oil. The 1H NMR spectrum (Table 1) was very similar to that of *pongaglabrone* (**5**), except for an ABX system characteristic of H-2, H-3 *ax* and H-3 *eq* in a flavanone skeleton. Its mass spectrum gave a parent ion at [M^+ , m/z 308], with fragments **8a** [$m/z = 160$] and **9a** [$m/z = 148$] originating from C ring RDA cleavage. Additionally, the fragment **9b** [$m/z = 149$] correspond to pathway II cleavage of the flavonoid skeleton (Fig. 3).

Compound **10** was obtained as colourless needles. The 1H NMR spectrum (Table 1) displayed absorptions for the hydrogens of A and B rings comparable to those of **8**. This time, however, two doublets at δ 5.03 (1H, *d*, $J = 9.3$ Hz) and δ 4.73 (1H, *d*, $J = 7.5$ Hz), together with a double doublet at δ 3.71 (1H, *dd*, $J = 7.5$ and 9.3 Hz), corresponded to H-2, H-4 and H-3 absorptions of a 3,4-dioxygenated flavan. Signals at δ 3.00 (3H) and δ 3.60 (3H) showed that **10** was a 3,4-dimethoxyflavan derivative. The coupling constants observed for H-2 and H-4 are very close to those reported in the literature (Harborne et al., 1975) for the 2:3-*trans*-3:4-*trans* configuration. This was confirmed further by the NOE data obtained by irradiation of H-2 and H-4 (Fig. 4), which also permitted the correct assignment of the chemical shifts for H-2, H-4 as well as for each methoxyl group. The ^{13}C NMR spectrum (see Section 3) and DEPT (90 and 135°) analysis gave signals corresponding to five quaternary carbons, 12 methines and 2 methyl groups. Signals at δ 80 (C-2), δ 81.98 (C-3) and δ 79.86 (C-4) confirm the presence of a 3,4-dioxygenated flavan skeleton. HR-EIMS gave a molecular ion at $M^+ 310.1196$ corresponding to $C_{19}H_{18}O_4$, with the most prominent mass spectral fragment corresponding to **10a** [$m/z = 176$], and **8b** [$m/z = 134$ RDA cleavage of C ring] and **8c** [$m/z = 91$] (Fig. 3).

The dichloromethane extract of *Lonchocarpus latifolius* roots on chromatographic analysis (column and prep TLC) also led to isolation of *medicarpin* **13** (Fig. 1) identified by comparing its spectral data with literature values (Afzail and Al-Orquat, 1986).

The petrol, dichloromethane and methanol extracts obtained from roots of *L. latifolius* were also submitted to the brine shrimp lethality (Table 2) (Meyer, 1982) and bioautography tests (Magalhães et al., 1998). The petrol and dichloromethane extracts were active against *Bacillus subtilis*, *Aspergillus niger* and *Rhizopus oryzae*, whereas compound **11** was active against *B. subtilis*, *A. niger* and *Cladosporium cladosporioides* (Magalhães et al., 1998). The brine shrimp lethality and the insecticidal activity (World Health Organization No. 443, 1970) results are noted, respectively in Tables 3 and 4.

The mean oxidation vs methylation values (O/M) of the flavonoids in the petrol extracts from the roots of *L. subglaucescens* (Magalhães et al., 1996) and *L. latifolius* suggest that *L. subglaucescens* is more highly evolved than *L. latifolius* as deduced previously on the basis of

Table 1
¹H NMR spectral data (300 MHz, CDCl₃) for compounds **8**, **9** and **10**

	8	9	10
H-2	5.50 (1H, <i>d</i> , <i>J</i> = 10)	5.53 (1H, <i>dd</i> , <i>J</i> = 3; 13) 2.90 <i>eq</i> (1H, <i>dd</i> , <i>J</i> = 3; 17)	5.03 (1H, <i>d</i> , <i>J</i> = 9.3)
H-3	4.20 (1H, <i>d</i> , <i>J</i> = 10)	3.10 <i>ax</i> (1H, <i>dd</i> , <i>J</i> = 13; 17)	3.71 (1H, <i>dd</i> , <i>J</i> = 7.5; 9.3)
H-4	—	—	4.73 (1H, <i>dd</i> , <i>J</i> = 7.5; 9.3)
H-5	7.88 (1H, <i>d</i> , 9)	7.89 (1H, <i>d</i> , <i>J</i> = 9)	7.34 (1H, <i>d</i> , <i>J</i> = 9)
H-6	7.23 (1H, <i>dd</i> , <i>J</i> = 1; 9)	7.20 (1H, <i>dd</i> , <i>J</i> = 1; 9)	7.18 (1H, <i>dd</i> , <i>J</i> = 1; 9)
H-2'	7.54 (2H, <i>dd</i> , <i>J</i> = 2; 8)	7.05 (1H, <i>d</i> , <i>J</i> = 2)	—
H-3'	—	—	—
H-4'	7.4–7.5 (3H, <i>m</i>)	—	7.4–7.5 (5H, <i>m</i>)
H-5'	—	6.87 (1H, <i>d</i> , <i>J</i> = 8)	—
H-6'	7.54 (2H, <i>dd</i> , <i>J</i> = 2; 8)	6.97 (1H, <i>dd</i> , <i>J</i> = 2; 8)	—
H-2''	7.62 (1H, <i>d</i> , <i>J</i> = 2)	7.60 (1H, <i>d</i> , <i>J</i> = 2)	7.56 (1H, <i>d</i> , <i>J</i> = 2)
H-3''	6.93 (1H, <i>dd</i> , <i>J</i> = 1; 2)	6.93 (1H, <i>dd</i> , <i>J</i> = 1; 2)	6.81 (1H, <i>dd</i> , <i>J</i> = 1; 2)
H-1'''	—	6.03 (2H, <i>s</i>)	—
OCH ₃	3.42 (3H, <i>s</i>)	—	3.00 (3H, <i>s</i>)
OCH ₃	—	—	3.60 (3H, <i>s</i>)

^d = Doublet; *dd* = double doublet; *m* = multiplet; *s* = singlet; *J* = coupling constant in Hertz.

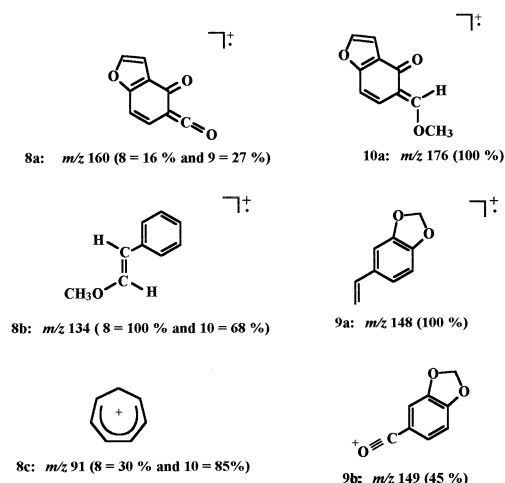


Fig. 3. Fragment ions corresponding to prominent peaks observed in the mass spectra of compounds **8**, **9** and **10**.

morphological criteria (Table 5). The (O/M) values of each species of flavonoids has been considered as an indirect expression of the corresponding redox potential and has been claimed as an useful approach for analyzing evolution trends in *Lonchocarpus*–*Derris* complex (Gottlieb, 1972).

HPLC analysis of petrol dichloromethane and methanol extracts of roots from the same plant collected in August 1994 and 1996 showed a very similar chromatographic profile; however, only the dichloromethane and methanol extracts of the 1994 collection contained medicarpin (**13**).

HPLC of the petrol extract from the stems leaves and seeds collected in February 1997 showed that the petrol extract from leaves and seeds consisted mainly of lanceolatin B (**3**) and 8-(α,β -dimethylallyl)-pongamol (**11**),

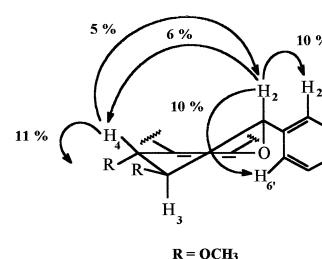


Fig. 4. NOE differential spectrum data of **10**, enhancements observed by irradiation of H-2 and H-4.

Table 2

Brine shrimp lethality test results for extracts from *Lonchocarpus latifolius* roots and compound **11**

Preparation	LC 50 ($\mu\text{g/ml}^{-1}$)
Petrol extract	0.1
CH ₂ Cl ₂ extract	6.98
MeOH extract	9.25
Compound 11	2.69
Control (H ₂ O)	> 1000

while that of stems did not exhibit peaks corresponding to pongaglabrone (**5**) and (**10**).

Karanjin (**2**), lanceolatin B (**3**) and 8-(α,β -dimethylallyl)-pongamol (**11**) were the most abundant flavonoids in the petrol extract of root samples collected in 1994 and in 1996 (Tables 6 and 7), while 8-(α,α -dimethylallyl)-pongamol (**11**) was the main flavonoid in the petrol extracts of stems, leaves and seeds (Table 7). The highest concentration of medicarpin (**13**) was observed in the methanol extract from root samples collected in 1994 (Table 6).

Table 3
Insecticidal test with *Aedes aegypti* for extracts from *Lonchocarpus latifolius* roots

Extract	Solvent	Concentration (ppm)	No. of larvae	No. of larvae died	% Mortality	CL 50 (ppm)	CL 90 (ppm)
MLR ^a	Methanol	5.6	200	45	22.5	10.89	86.88
		13	200	118	59.0		
		24	250	202	80.8		
EPLR	Acetone	0.001	75	26	34.7	0.0043	13.3
		0.01	125	73	58.4		
		0.1	174	144	82.3		
Control	Acetone	–	475	3	0.6		
Control	Methanol	–	225	2	0.9		
Control	H ₂ O	–	375	3	0.8		

^a MLR, methanol extract; EPLR, petrol extract.

Table 4
Mean oxidation vs methylation values (O/M) from *Lonchocarpus latifolius* and *Lonchocarpus subglaucescens*

Species	O/M
<i>Lonchocarpus latifolius</i>	+ 1.75/ + 1.33
<i>Lonchocarpus subglaucescens</i>	+ 2.8/ + 2.1

Table 5
Flavonoid contents of extracts of *Lonchocarpus latifolius* roots collected in August 1994

Flavonoid	Flavonoid contents in roots (mg/g) extracts		
	Petrol	Dichloromethane	Methanol
1	2.13	–	–
2	0.90	–	–
3	2.02	–	–
4	0.39	–	–
5	0.72	–	–
6	0.70	–	–
10	0.16	–	–
11	8.05	–	–
13	–	1.62	7.03

Seasonal analysis made by HPLC of petrol extracts from root samples of one plant collected in August 1996 (winter), as well as September 1996 (spring) and February 1997 (summer) showed that the highest concentration of flavonoids occurred in the spring. 8-(α,β -Dimethylallyl)-pongamol (**11**) was by far the most abundant flavonoid; its concentration was not significantly affected by the season, while 3,4-dimethoxyflavan (**10**) was the least abundant among the nine quantified flavonoids (Table 7).

3. Experimental

3.1. Plant material

Roots of *Lonchocarpus latifolius* were collected at Santa Elisa Farm of the Instituto Agronômico de Campinas, in

Table 6
Seasonal variation of flavonoids in *Lonchocarpus latifolius* roots (petrol extract)

Flavonoid	Flavonoid content in roots (mg/g)		
	August 1996 (Winter)	November 1996 (Spring)	February 1997 (Summer)
1	0.47	0.87	0.12
2	0.27	0.92	0.26
3	0.46	1.95	0.66
4	0.00	0.26	0.09
5	0.20	0.31	0.12
6	0.18	0.39	0.12
10	0.04	0.13	0.03
11	1.86	2.30	1.61

Table 7
Flavonoid contents in stems, leaves and seeds of *Lonchocarpus latifolius* collected in 1997 (petrol extract)

Flavonoid	Flavonoid contents (mg/g)		
	Stems	Leaves	Seeds
1	0.40	–	–
2	0.67	–	–
3	0.29	1.08	0.33
4	0.21	–	–
5	–	–	–
6	0.32	–	–
10	–	–	–
11	1.48	3.67	1.02

August 1994, August 1996, November 1996 and February 1997. Voucher specimens were deposited at the herbarium (UEC 21974) of Campinas State University, Campinas-SP, Brazil.

3.2. General extraction and isolation procedure

Dried roots (714 g) of *L. latifolius* were successively extracted with petrol (30–60°C), CH₂Cl₂ and MeOH for

60 h in a Soxhlet apparatus. After solvent evaporation, the petrol extract was a viscous yellow oil (12.5 g), whereas the dichloromethane extract (7.5 g) and the methanol extract (43 g) were brown gums. A portion of the petrol extract (6 g) was subjected to 240 g silica gel chromatography, eluted first with petrol. The eluent polarity was then gradually increased by addition of CH_2Cl_2 , EtOAc and MeOH to furnish 283 fractions (200 ml each) which were reduced to 43 fractions following TLC analysis. Most of the compounds of interest were present in 12 groups ranging from fractions 56 to 156. Each fraction was further submitted to prep. TLC, eluted with petrol–EtOAc 8:2 v/v permitting the isolation of **1** (14 mg), **2** (155 mg), **3** (38 mg), **4** (7 mg), **5** (8 mg), **6** (5 mg), **7** (3 mg), **8** (2 mg), **9** (3 mg), **10** (5 mg), **11** (200 mg) and **12** (3 mg), respectively.

3.3. Compound **7** (3,5-dimethoxy-2'',2''-dimethylpyrano-(5'',6'':8,7)-flavone)

Viscous yellowish oil. ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 1.45 (6H, s, CH_3), 3.87 (3H, s, OCH_3), 3.88 (3H, s, OCH_3), 5.79 (1H, d, $J=10$ Hz, H-3''), 6.48 (1H, s, H-6), 6.89 (1H, d, $J=10$ Hz, H-4''), 7.5–7.6 (3H, m H-3', H-4' and H-5'), 8.03 (2H, d, $J=8$ Hz, H-2' and H-6').

3.4. Compound **8** (3-methoxy-(2'',3'':7,8)-furanoflavanone)

Colourless amorphous powder. UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (log ϵ) 240 (4.74). HR-EIMS, m/z found 294.0894; calcd. 294.0892 for $\text{C}_{18}\text{H}_{14}\text{O}_4$; EIMS m/z (rel. int.): 294 (9); 160 (16); 134 (100); 105 (4); 91 (30); 77 (9). ^1H NMR (Table 1).

3.5. Compound **9** (3,4-methylenedioxy-(2'',3'':7,8)-furanoflavanone)

Viscous yellowish oil. UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (log ϵ) 238 (4.61). IR ν_{max} cm^{-1} (KBr): 2922, 2852, 1700, 1678, 1459, 1089. EIMS m/z (rel. int.) 308 (20); 160 (18); 149 (13); 148 (100); 132 (4). ^1H NMR (Table 1).

3.6. Compound **10** (2,3-trans-3,4-trans)-3,4-dimethoxy-(2'',3'':7,8)-furanoflavanone)

Needles, melting point: uncorr. 81.9–82.2°C. $[\alpha]_{20}^{\text{D}} = +21^\circ$ ($c=0.4$ CH_2Cl_2). UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (log ϵ) 248 (4.43). IR ν_{max} cm^{-1} (KBr): 2926, 2856, 2925, 1622, 1474, 1142, 1067. HR-EIMS, m/z found 310.1196; calcd. 310.1205 for $\text{C}_{19}\text{H}_{18}\text{O}_4$; EIMS m/z (rel. int.): 310 (2), 176 (100), 161 (64), 147 (47), 134 (68), 134 (105 (15), 91 (85), 77 (38). ^1H NMR (Table 1). ^{13}C NMR (75 MHz, CDCl_3) δ : C-2 (80.50), C-3 (81.96), C-4 (79.86), C-5 (105.15), C-6 (124.19), C-7 (156.04), C-8 (116.70), C-9 (147.90), C-10 (115.36), C-1' (138.13), C-2' (127.19), C-3' (128.36), C-4' (128.44), C-5' (128.36), C-6' (127.19), C-2'' (144.00), C-3'' (104.09), OCH_3 (57.79; 59.53).

3.7. HPLC analysis

Before use as a standard, each isolated flavonoid was purified by semi-preparative HPLC (UV detection; $\lambda=240$ nm), using an ODS column (250 mm \times 10 mm i.d.) with a solvent gradient of $\text{H}_2\text{O}-\text{CH}_3\text{CN}$ (of 0–100% CH_3CN ; 60 min; 1.0 ml min^{-1}). An aliquot (2 μl) of each extract (petrol extract from roots, stems, seeds and leaves and dichloromethane extract from roots) was analysed by HPLC (UV detection; $\lambda=240$ nm) using an ODS column (100 mm \times 4.6 mm i.d.) eluted with a $\text{H}_2\text{O}-\text{CH}_3\text{CN}$ gradient (20–80% CH_3CN ; 30 min; 0.8 ml min^{-1}). The peaks in the chromatograms were identified by co-injection of each standard compound mentioned above, being found the R_T (in min) for **1** (19.08); **2** (15.27); **3** (14.08); **4** (18.23); **5** (13.61); **6** (14.71); **10** (17.52); **11** (18.84); **13** (11.30), respectively.

An aliquot (2 μl) of the methanol extract was also analysed by using an ODS column (100 mm \times 4.6 mm i.d.) eluted with a solvent gradient of $\text{H}_2\text{O}-\text{MeOH}$ (3:97–0:100; 30 min; 0.8 ml min^{-1}).

Quantification of flavonoids in the extracts was made through the method of external calibration with correlation coefficients of 0.999.

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