LONCHOCARPENE, A STILBENE, AND LONCHOCARPUSONE, AN ISOFLAVONE: TWO NEW PYRANOPOLYPHENOLS FROM *LONCHOCARPUS NICOU* ROOTS

MOURAD KAOUADJI, AMEGNINOU AGBAN, ANNE-MARIE MARIOTTE,

Laboratoire de Pharmacognosie, UFR de Pharmacie, Université Scientifique et Médicale de Grenoble, Domaine de La Merci, F-38700 La Tronche, France

and MICHEL TISSUT

Laboratoire de Physiologie Cellulaire Végétale, UFR de Biologie, Université Scientifique et Médicale de Grenoble, BP 68, F-38400 Saint Martin d'Hères, France

ABSTRACT.—Among apolar constituents of *Lonchocarpus nicou* roots (Leguminosae), two new pyranopolyphenols occur in the *n*-hexane extract, along with rotenoids, the main components. Structures of lonchocarpene (**1**), a stilbene, and lonchocarpusone (**3**), an isoflavone, were determined by spectroscopic evidence.

With its 100-150 species growing in the tropics and the southern hemisphere, *Lonchocarpus* is a genus belonging to the large subfamily Lotoideae of the Leguminosae (1). It produces a variety of secondary phenolic metabolites among which are rotenoids. During investigation of apolar polyphenols in the roots of a Peruvian member, *Lonchocarpus nicou* Benth., eight compounds have been isolated and characterized from the *n*-hexane extract: six rotenoids (deguelin, deguelinenol, tephrosin, rotenone, rotenonenol, and 12a-hydroxyrotenone) and two new minor constituents named lonchocarpene (**1**) and lonchocarpusone (**3**). As only rotenone and deguelin have been previously identified in this species (2), isolation of the hydroxyrotenoids will be published elsewhere; we now wish to report the identification of the two new natural products, on the basis of their ms, ^{1}H- and ^{13}C-nmr data.

Viscous, lonchocarpene (**1**), $C_{21}H_{22}O_3$, $M^{+\cdot}$ *m/z* 322, characterized by a blue fluorescence, was the least polar constituent of the *L. nicou* roots *n*-hexane extract. Its ^{1}H-nmr spectrum (CDCl$_3$; 300 MHz) indicated two uncoupled Me groups at δ 1.44 ppm, two OMe groups at δ 3.82 ppm, a *cis*-ethylenic chain at δ 5.65 and 6.35 ppm, d, $J=10$ Hz, a *trans*-ethylenic chain at δ 6.84 and 7.03 ppm, d, $J=16$ Hz, a 1,2,4-trisubstituted aromatic ring on which protons are located at δ 7.26 ppm, dd, $J=7$, 2 Hz; δ 7.15 ppm, d, $J=2$ Hz; and δ 6.77 ppm, d, $J=7$ Hz, and a 1,3,5-trisubstituted aromatic ring with two very nearly equivalent protons at δ 6.63 ppm, *meta*-coupled with one at δ 6.37 ppm, dd, $J=2$, 2 Hz.

To explain the two proton signals at δ 6.63 ppm, the 1,3,5-trisubstituted aromatic ring must bear the two methoxy groups (δ 3.82 ppm), the third substitution being identified with the *trans*-ethylenic chain (δ 6.84 and 7.03 ppm) connecting the two aromatic rings. The second phenyl was 1,2,4-trisubstituted by this double bond as well as by a *cis*-ethylenic system (δ 5.65 and 6.35 ppm) included in a *gem*-dimethylpyran, the equivalent methyls of which are at δ 1.44 ppm in the ^{1}H-nmr spectrum. The tetrasubstituted 0-bound C-atom is observed at δ 77.5 ppm in the ^{13}C-nmr spectrum. Between the two possibilities, **1** and **2**, of substitution relative to this aromatic ring by the dimethylpyran, structure **2** was ruled out on the basis of H-2′ deshielding (δ 7.15 ppm) in comparison with H-2 and H-6 (δ 6.63 ppm) which are affected by shielding induced by *ortho*-0-substitution and increased by *para*-0-substitution. This is also confirmed by the uv absorption at λ 312 nm only compatible with structure **1**. Consequently, lonchocarpene was considered to be 2″,2″-dimethylpyran (5″,6″: 3′,4′) 3,5-dimethoxystilbene, a new natural product. The ^{13}C-nmr spectrum was in agreement with the pro-

posed structure. Carbon 1' (δ 130.0 ppm) was naturally shielded by O-substitution in the *para*-position contrary to C-1 (δ 139.8 ppm).

Amorphous, mp 139-141°, lonchocarpusone (**3**), $C_{23}H_{22}O_6$, $M^{+\cdot}$ *m/z* 394, was more polar on silica gel than the major constituents of the *n*-hexane extract, the rotenoids and their hydroxy derivatives. Its ^{1}H-nmr spectrum (CDCl$_3$; 300 MHz) exhibited signals corresponding to two uncoupled Me groups at δ 1.49 ppm, three OMe at δ 3.77, 3.85, and 3.92 ppm, a *cis*-ethylenic chain at δ 5.72 and 6.85 ppm, d, *J*=8.8 Hz, an isolated H at δ 7.96 ppm, an aromatic ring with two *ortho*-coupled protons at δ 8.04 and 6.82 ppm, d, *J*=8.8 Hz, and an aromatic ring carrying two uncoupled *para* protons at δ 6.95 and 6.62 ppm.

1

2

3

4
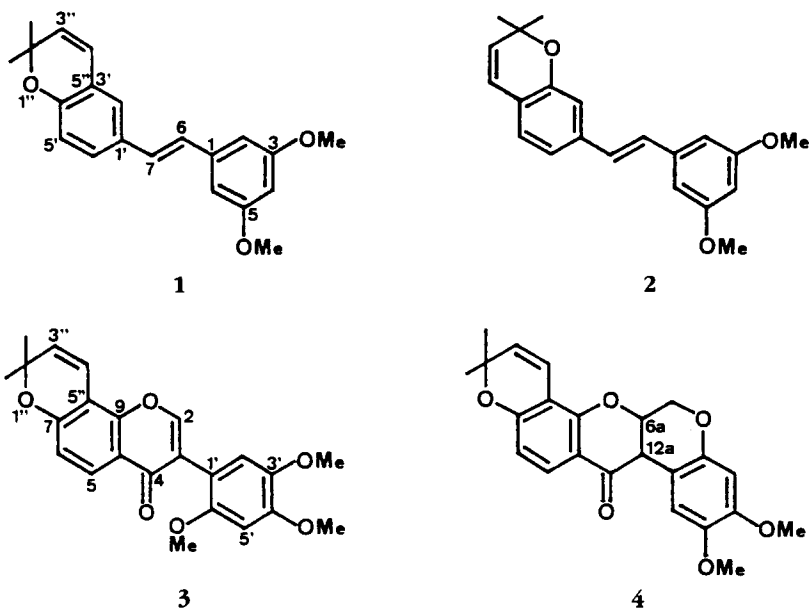
As in the case of lonchocarpene (**1**), a *gem*-dimethylpyran was detected in lonchocarpusone (**3**) by ^{1}H nmr (see above) and confirmed by analysis of the ^{13}C-nmr spectrum which displayed the quaternary O-bound C-atom at δ 77.6 ppm. The low field region of this spectrum showed seven additional signals corresponding to a carbonyl function at δ 175.8 ppm, five quaternary ethylenic O-bound carbons at δ 157.2, 152.4, 152.0, 149.9, and 143.3 ppm, along with an ethylenic O-bound methine at δ 154.0 ppm. Consequent to ^{1}H shielding occurring in C_6D_6 for the three OMe (-0.40 ppm$\leqslant\Delta\delta\leqslant-0.37$ ppm), each group had to be adjacent to an aromatic H (3, 4). On the other hand, existence of the fragmentation pattern starting from the molecular ion *m/z* 394↦*m/z* 379↦*m/z* 351, by the successive loss of a methyl radical then a mole of CO (see Scheme 1), proved that two OMe groups were either *ortho*- and/or *para*-related (5). From those data, it was clearly established that those groups can only be attached to the 1,2,4,5-tetrasubstituted aromatic ring by three of the five quaternary ethylenic O-bound C-atoms listed above; the remaining two carbons were therefore attached to the 1,2,3,4-tetrasubstituted aromatic ring; one of them (δ 157.2 ppm) was included in the dimethylpyran and the other (δ 152.4 ppm) in a γ-pyrone ring along with the ethylenic O-bound methine (δ 154.0 ppm) and the CO group responsible for deshielding of the *ortho*-related H (δ 8.04 ppm). Lonchocarpusone was finally identified as an isoflavone, the trimethoxyphenyl attached α to the CO, as shown in formula **3**, giving rise to the prominent peak at *m/z* 363 corresponding to (M-31)$^+$ due to loss of MeO-6' as indicated in Scheme 1. A comparative analysis of H-4" δ-values, 6.35 ppm in **1** vs 6.85
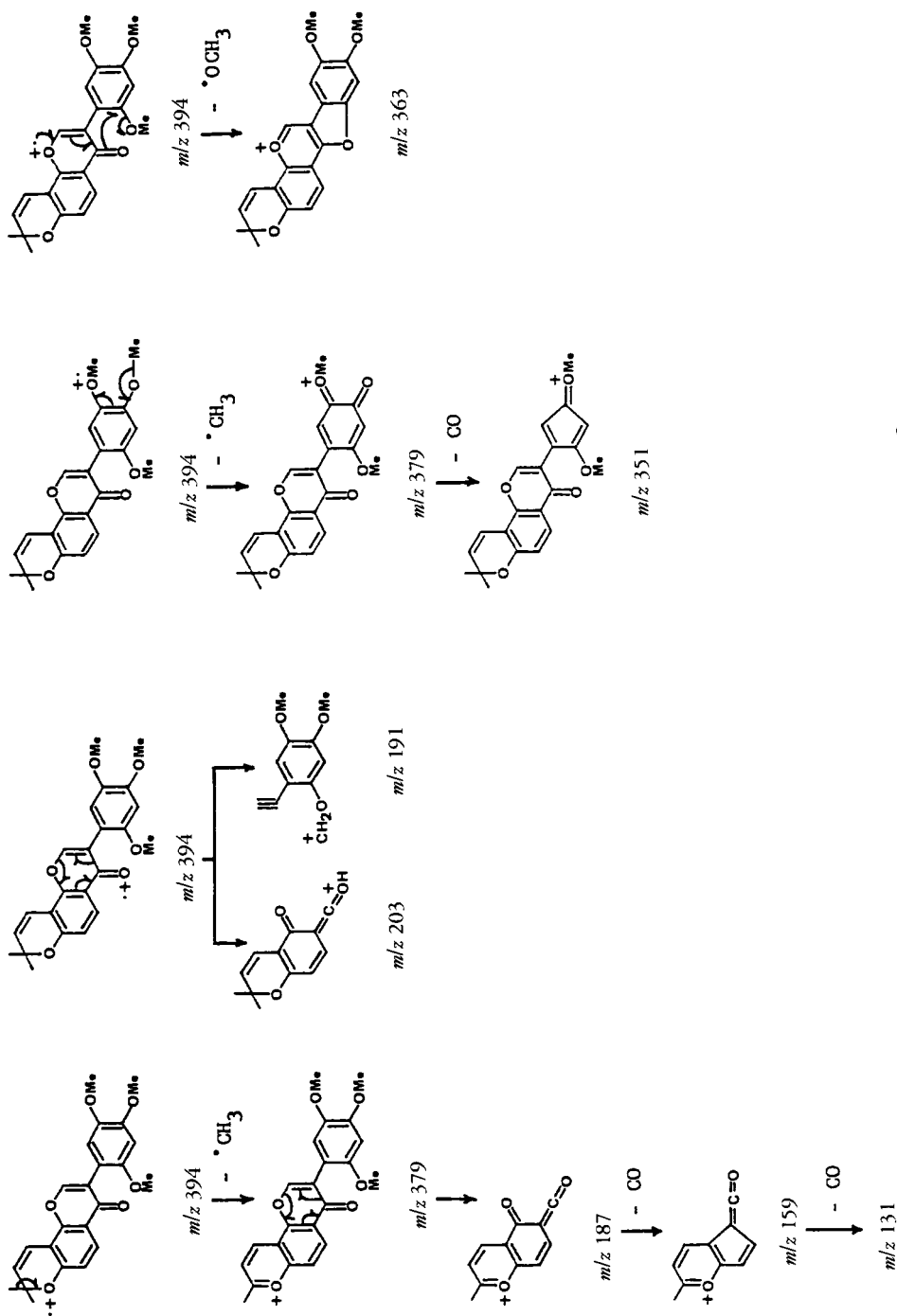
SCHEME 1.   Eims fragmentation of lonchocarpusone (3).

ppm in **3**, convincingly supported the proposed structure for lonchocarpusone in which this proton had to be close to an oxygen as in deguelin (**4**) where its signal is, in this case, at a slightly higher field (δ 6.67 ppm) on account of a lack of a double bond in the dihydropyrone ring. On the basis of the reported data, lonchocarpusone (**3**) was considered to be 2″,2″-dimethylpyran (5″,6″: 8,7) 3′,4′,6′-trimethoxyisoflavone, a new natural product.

One can notice that the stilbene and the isoflavone reported here exhibit a dimethylpyran ring, as deguelin, the main component present in the *n*-hexane extract of *L. nicou* roots.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Analytical and preparative tlc were carried out on silica gel 60 F-254 plates (E. Merck). Silica gel (70-230 mesh, E. Merck), cellulose microcrystalline (E. Merck), Sephadex LH-20 (Pharmacia Fine Chemicals) were used for column chromatography. Silica gel 60 PF-254 containing gypsum (E. Merck), for preparative layer chromatography, was used for circular centrifugal tlc. Hplc was carried out on a Waters Model 6000A instrument equipped with a variable wavelength detector and μBondapak C_{18} column (30 cm). Mps were obtained from a capillary melting point Büchi apparatus and were uncorrected. Uv spectra were measured in MeOH on a Beckman S25 spectrometer. ^{1}H- and ^{13}C-nmr spectra were recorded on an AM300 Brucker nmr spectrometer (CDCl$_3$ or C$_6$D$_6$; δ ppm/TMS). Extensive decoupling was used to verify assignments. Ei mass spectra were taken on an AEI MS902 mass spectrometer (70 eV).

PLANT MATERIAL.—*L. nicou* roots (1 kg) were obtained as a gift from the firm Sedagri-Rue de la Méditerranéenne, F-34300 Agde. A voucher specimen has been deposited at Laboratoire de Pharmacognosie de Grenoble-Domaine de La Merci, F-38700 La Tronche.

EXTRACTION AND FRACTIONATION.—Air-dried powdered roots of *L. nicou* roots (200 g) were extracted at room temperature with *n*-hexane (4 × 500 ml) to afford a pale yellow amorphous powder (10.3 g). A portion (3 g) was separated by column chromatography (SiO$_2$) eluted by a gradient from C$_6$H$_6$ to C$_6$H$_6$-MeOH (70:30). Combination of similar eluates on the basis of tlc analysis on silica gel in C$_6$H$_6$-MeOH (99:1) and *n*-hexane-CHCl$_3$-*i*PrOH-MeOH (95:3:1:1) provided thirty-five fractions. Lonchocarpene was detected in the two first fractions; deguelin and rotenone were found in fractions 6-17 accompanied with traces of tephrosin and 12a-hydroxyrotenone which were more concentrated in fractions 19 and 20. Deguelinenol and rotenonenol were located in fractions 25-28 whereas lonchocarpusone was the main uv absorbing constituent in fraction 32.

Purification of lonchocarpene was performed by subjecting fractions 1 and 2 (330 mg) to cctlc on silica gel (C$_6$H$_6$ to C$_6$H$_6$-MeOH, 90:10), followed by preparative tlc on silica gel with two developments in *n*-hexane-C$_6$H$_6$ (60:40). Elution of the upper band gave 76 mg of impure lonchocarpene filtered through a cellulose column using *n*-hexane. This procedure yielded 35 mg of the stilbene with which final purification was carried out on a portion (20 mg) by semipreparative hplc on reverse phase C$_{18}$ [MeOH-H$_2$O (80:20); flow rate 2 ml/min] providing pure lonchocarpene as a colorless oil (12 mg).

Fraction 32 (231 mg) of the silica column was used to isolate lonchocarpusone in four steps: by cctlc on silica gel (C$_6$H$_6$ up to C$_6$H$_6$-MeOH, 90:10), silica column (C$_6$H$_6$-MeOH, 95:5), Sephadex LH-20 column (MeOH) followed by cctlc on silica gel (*n*-hexane-C$_6$H$_6$, 50:50) to separate the isoflavone from several surrounding fluorescent materials. A further purification by crystallization in *n*-hexane was needed to obtain pure lonchocarpusone as a white amorphous powder (27 mg).

Lonchocarpene (**1**).—a colorless oil; uv λ MeOH 312, 270, 227 nm; ms *m/z* (%) 322 (M$^{+\cdot}$; 33), 307 (100), 249 (12), 171 (11), 153 (52), 139 (12), 116 (22), 115 (11), 104 (13), 91 (13), 89 (18), 77 (11); ^{1}H nmr (300 MHz; CDCl$_3$) δ 7.26 (1H, dd, *J*=7, 2 Hz, H-6′), 7.15 (1H, d, *J*=2 Hz, H-2′), 7.03 (1H, d, *J*=16 Hz, H-7 or 8), 6.84 (1H, d, *J*=16 Hz, H-7 or 8), 6.77 (1H, d, *J*=7 Hz, H-5′), 6.63 (2H, d, *J*=2 Hz, H-2 and 6), 6.37 (1H, dd, *J*=2, 2 Hz, H-4), 6.35 (1H, d, *J*=10 Hz, H-4″), 5.65 (1H, d, *J*=10 Hz, H-3″), 3.82 (6H, s, MeO-3 and 5), 1.44 (6H, s, Me-2″); ^{13}C nmr (75.5 MHz; CDCl$_3$) δ 161.0 (2C, C-3 and 5), 152.9 (C-4′), 139.8 (C-1), 131.1 (C-4″), 130.0 (C-1′), 128.9 (C-8 or 3″), 127.6 (C-8 or 3″), 126.5 (C-7), 124.4 (C-2′ or 6′), 122.2 (C-2′ or 6′), 121.3 (C-3′), 116.6 (C-5′), 104.4 (2C, C-2 and 6), 99.7 (C-4), 77.5 (C-2″), 55.3 (2C, MeO-3 and 5), 28.1 (2C, Me-2″).

Lonchocarpusone (**3**).—a white amorphous powder mp 139-141°; uv λ MeOH 317 sh, 292, 256, 226 nm; ms *m/z* (%) 394 (M$^{+\cdot}$; 100), 379 (86), 363 (40), 351 (13), 349 (30), 333 (6), 330 (5), 321 (10), 305 (3), 298 (2), 293 (2), 278 (3), 277 (3), 237 (6), 213 (15), 203 (34), 191 (40), 187 (35), 182 (28), 168 (32), 159 (9), 131 (13); ^{1}H nmr (300 MHz; CDCl$_3$) δ 8.04 (1H, d, *J*=8.8 Hz, H-5), 7.96 (1H, s, H-2), 6.95 (1H, s, H-2′), 6.85 (1H, d, *J*=8.8 Hz, H-4″), 6.82 (1H, d, *J*=8.8 Hz, H-6), 6.62 (1H, s, H-5′), 5.72

(1H, d, $J=8.8$ Hz, H-3″), 3.92 (3H, s, MeO), 3.85 (3H, s, MeO), 3.77 (3H, s, MeO), 1.49 (6H, s, Me-2″); /C_6D_6 δ 8.30 (1H, d, $J=8.8$ Hz, H-5), 7.62 (1H, s, H-2), 7.10 (1H, s, H-2′), 6.70 (1H, d, $J=8.8$ Hz, H-6), 6.65 (1H, d, $J=8.8$ Hz, H-4″), 6.49 (1H, s, H-5′), 5.21 (1H, d, $J=8.8$ Hz, H-3″), 3.55 (3H, s, MeO), 3.40 (3H, s, MeO), 3.37 (3H, s, MeO), 1.21 (6H, s, Me-2″); ^{13}C nmr (75.5 MHz; $CDCl_3$) δ 175.8 (C-4), 157.2 (C-7), 154.0 (C-2), 152.4 (C-9), 152.0 (C-6′), 149.9 (C-4′), 143.3 (C-3′), 130.2 (C-4″), 126.8 (C-5), 121.6 (C-3), 118.5 (C-10), 115.7 (C-6 or 2′ or 3″), 115.1 (2C, C-6 and/or 2′ and/or 3″), 112.5 (C-1′), 109.3 (C-8), 98.7 (C-5′), 77.6 (C-2″), 57.0 (MeO), 56.7 (MeO), 56.3 (MeO), 28.1 (2C, Me-2″).

ACKNOWLEDGMENTS

LITERATURE CITED

1. V.H. Heywood, in: "Chemotaxonomy of the Leguminosae." Ed. by J.B. Harborne, D. Boulter, and B.L. Turner, chap. 1. Academic Press, London, 1971, pp. 1-29.
2. N.E. Delfel, *J. Assoc. Off. Anal. Chem.*, **56**, 1343 (1973); *Chem. Abstr.*, **80**, 67260e (1974).
3. R.G. Wilson, J.H. Bowie, and D.H. Williams, *Tetrahedron*, **24**, 1407 (1968).
4. K.R. Markham and T.J. Mabry, in: "The Flavonoids." Ed. by J.B. Harborne, T.J. Mabry, and H. Mabry, chap. 2. Chapman and Hall, London, 1975, pp. 45-77.
5. D.H. Williams and I. Fleming, "Spectroscopic Methods in Organic Chemistry," McGraw Hill, London, 1973, p. 167.