

Substituted tubaic acids, new oxidative rotenoid metabolites from *Lonchocarpus nicou*

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Abstract—A lipophile extract of *Lonchocarpus nicou* roots afforded two likely *O*-substituted isomers of the known tubaic acids. Spectroscopic analysis assigned to the former, named (–)-rotoic acid, the new 4-(2,3-dihydro-6,7-dimethoxychromon-3-oxy) tubaic acid structure which differs from (–)-deguoic acid, the latter, by the β -tubaic acid part. Biogenetically, the two compounds could be considered as resulting from the parent rotenone and deguelin, respectively, by oxidative ring-C cleavage.

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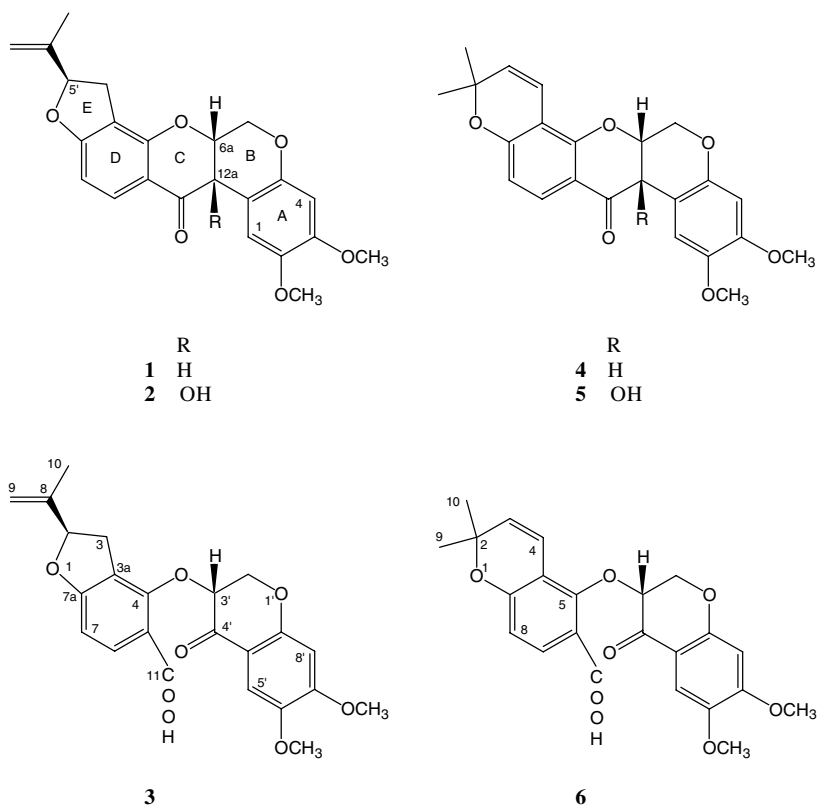
Likely, the four closely related tropical genera belonging to the tribe *Millettieae* of the Fabaceae (*Derris*, *Millettia*, *Mundulea* and *Tephrosia*), *Lonchocarpus* produces numerous diversified polyphenolics based upon isoflavonoid skeleton.^{1–12} Occurrence of such compounds in *Lonchocarpus nicou* (Aublet) D.C. roots is in favour of the four main rotenoids: (–)-rotenone (**1**) and (–)-deguelin (**4**) along with their (–)-12a β -hydroxy derivatives **2** and **5**, thus contrasting with all the other minor phytochemicals.^{13,14} Treatment of the defatted benzene extract¹⁵ (24 g) of *L. nicou* roots (200 g) by multistep chromatographic techniques led to the isolation of the two new isomers **3** (25 mg) and **6** (18 mg) derived from the known tubaic acids.^{16,17} Their structures were established by spectroscopic evidence including UV, MS as well as NMR.

Isolated as a white amorphous powder, $[\alpha]_{\text{D}}^{23}$ –309 (*c* 0.01, C₅D₅N), compound **3** exhibited a UV spectrum consistent with two bands at λ_{max} (MeOH): 340 and 274 nm, respectively, as well as the molecular formula C₂₃H₂₂O₈ deduced from HRESMS (found: 427.1404; calcd: 427.1393). The 13 double-bond equivalent from

the molecular formula was in agreement with the recorded ¹³C NMR data for 16 sp² C atoms (189 ppm > δ > 101 ppm) along with seven aliphatic carbons (88 ppm > δ > 17 ppm). Besides the four ¹H NMR signals in the shift range 8.5–6.5 ppm appropriate for benzenoid protons of which two exhibit *ortho*-coupling constant, indicating two benzene rings in this molecule (Table 1), the remaining 5 degrees of unsaturation are consistent with: two α,β -unsaturated carbonyl groups (δ 188.2 and 168.4 ppm), one isolated *gem*-disubstituted double bond (δ 144.8 and 112.8 ppm) with two non-equivalent protons (δ 5.07 and 4.84 ppm), and finally two different –CH₂–CH< fragments each belonging to a heterocycle. Of the two aromatic rings, one is 1,2,3,4-tetrasubstituted (AM system at δ 8.34 and 6.83 ppm and *ortho*-coupling of 8.4 Hz); the other is 1,2,4,5-tetrasubstituted as shown by the two singlets integrating for one proton at δ 7.59 and 6.69 ppm. In each aromatic ring, deshielding of the proton (δ 8.34 and 7.59 ppm) is involved by an electron-withdrawing substituent consisting in an *ortho*-CO group; inversely the remaining protons (δ 6.83 and 6.63 ppm) are shielded by two electron-donating OR substituents. The substitution pattern of the benzene rings was corroborated by the HMBC cross-peaks analysis,¹⁸ which furthermore pointed out *O*- and *C*-substituents for each one. The proton at δ 8.34 ppm (H-6) exhibited three large connectivities with quaternary *O*-bonded carbons: C-4

Keywords: Fabaceae; *Lonchocarpus nicou*; Rotoic acid; Deguoic acid; Opened rotenoids ring-C; *seco*-Rotenoids.

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**Table 1.** ^1H (400 MHz) and ^{13}C (100 MHz) NMR data for compounds **3** and **6** in $\text{C}_5\text{D}_5\text{N}$ (δ ppm; J Hz)

Compound 3				Compound 6			
H/C	^{13}C	^1H	HMBC	H/C	^{13}C	^1H	HMBC
<i>Tubaic acid moiety</i>							
2	87.8	5.34, br t (8.6)	C-9	2	77.3		
3	33.2	3.65, dd (15.9; 9.8) 3.19, br dd (15.9; 7.6)	C-2, C-3a, C-4, C-7a, C-8	3	130.6	5.54, d (10.0)	C-2, C-4a
3a	122.3			4	118.1	6.84, br d (10.0)	C-2, C-8a
4	156.8			4a	117.5		
5	118.1			5	155.6		
6	135.2	8.34, d (8.4)	C-4, C-7a, C-11	6	118.2		
7	106.0	6.83, d (8.4)	C-3a, C-5, C-7a	7	134.1	8.27, d (8.6)	C-5, C-8a, C-11
7a	165.3			8	113.2	6.87, br d (8.6)	C-4a, C-6, C-8a
8	144.5			8a	158.3		
9	112.7	5.07, br s 4.84, br s	C-2, C-10	9, 10	28.2, 28.1	1.41, s, 1.35, s	C-2, C-3
10	17.3	1.64, br s	C-2, C-8, C-9				
11	168.4	ND		11	168.3	ND	
<i>2,3-Dihydrochromonyl moiety</i>							
2'	71.6	5.22, dd (12.0; 6.4) 5.02, dd (12.0; 3.7)	C-3', C-4', C-8'a	2'	71.7	5.31, dd (13.4; 5.0) 4.91, dd (13.9; 5.4)	C-3', C-4', C-8'a
3'	79.6	5.43, dd (6.3; 3.8)	C-4, C-2', C-4'	3'	80.4	5.33, br t (5.1)	C-5, C-4', C-8'a
4'	188.2			4'	187.0		
4'a	112.8			4'a	113.0		
5'	108.0	7.59, s	C-4', C-6', C-7', C-8'a	5'	108.1	7.52, s	C-4', C-6', C-7', C-8'a
6'	146.1			6'	146.1		
7'	157.7			7'	157.7		
8'	101.2	6.69, s	C-4', C-4'a, C-6', C-7', C-8'a	8'	101.2	6.71, s	C-4'a, C-6', C-7', C-8'a
8'a	159.0			8'a	158.9		
6'-OCH ₃	56.3	3.70, s	C-6'	6'-OCH ₃	56.3	3.69, s	C-6'
7'-OCH ₃	56.5	3.75, s	C-7'	7'-OCH ₃	56.5	3.78, s	C-7'

ND: not detected.

(δ 156.8), C-7a (δ 165.3) and C-11 (δ 168.4), in agreement with 3J couplings. The latter C atom was identified with a carboxyl group where missing H in C_5D_5N was however recorded at δ 10.81 ppm in $CDCl_3$ and δ 12.38 ppm in $DMSO-d_6$. On the same ring, the proton at δ 6.83 ppm (H-7) showed only two large cross-peaks with two low field quaternary C-substituted positions C-5 (δ 118.1) and C-3a (δ 122.3). A third and weak connectivity was displayed by this proton with the previous C-7a (δ 165.3), according to 2J coupling. All these findings evidenced a 2,4-di-*O*- and 3-*C*-substituted benzoic acid. The remaining C-substitution was an endocyclic methylene group (δ 33.2) attached to a methine oxy (δ 87.8) as shown by the splitting pattern couplings of the corresponding H at δ 3.65 and 3.19 ppm for the *gem* nuclei ($J = 15.9$ Hz) and δ 5.34 ($J = 8.6$ Hz) for the only vicinal H-2. The doublet of doublets ($J = 9.8$ and 7.6 Hz) required for the latter nucleus was replaced by a broad triplet ($J = 8.6$ Hz) involved by long range coupling with the olefinic proton at δ 4.84 ppm of an isopropenyl side chain (C-8: δ 144.5, H_2C -9: δ 112.7 and H_3C -10: δ 17.3). Thereby, the benzoic acid C-substitution was a cyclized C_5 chain leading to a 2-isoprenyl-2,3-dihydrofuran joined to the aromatic. Ring closure of the heterocycle would occur at either the *ortho*- or the *para*-position with respect to the carboxy group. Although neither C-4 (δ 156.8) nor C-7a (δ 165.3) displayed any connectivity with H-2 in the HMBC spectrum, cyclization was deduced to locate at C-7a since C-4 exhibited a cross-peak with a methine oxy (δ 5.43) belonging to another chain of this molecule. Hence, the partial structure issued from the above results was a 4-*O*-substituted 2-isoprenyl-2,3-dihydrobenzofuran-5-carboxylic acid derived from tubaic acid.^{16,17}

The remaining C_{11} moiety attached to the tubaic acid part corresponded to six double-bond equivalent and was constituted by the next 1,2,4,5-tetrasubstituted benzene bearing two methoxy groups (δ 56.3 and 56.5), one methylene oxy (δ 71.6) where two non-equivalent protons (δ 5.22 and 5.02) were coupled with the previously reported nucleus (δ 5.43) of the methine oxy (δ 79.6) linked to both tubaic acid part as well as to the second conjugated carbonyl group (δ 188.2) of this compound. From this, 6,7-dimethoxy-2,3-dihydrochromon with 3-*O*-substitution followed. To conclude, the complete structure for this product was 4-(6,7-dimethoxy-2,3-dihydrochromon-3-oxy) tubaic acid (**3**) for which the trivial name (–)-rotoic acid has been assigned. This unusual new structure is unprecedented in the natural isoflavonoids derivatives in that it has a carboxyl group. It represents a fully oxidized version of the C(12)–C(12a) rotenone bond only involving ring-C opening with unaltered 2*R*- and probable 3'*R*-configuration as indicated by the negative optical activity for both compounds as well as for tubaic acid.¹⁷ The decisive clue for supporting the above mentioned stereochemistry at C-3' was given by the CD spectrum of (–)-rotoic acid. Similar to those of closely related structures as (3*R*)-isoflavanones¹⁹ and particularly to that of laevorotatory (3*R*)-sophorol,²⁰ it likely, with respect to the substitution pattern, exhibited a negative Cotton effect at ca. 310 nm (longest aromatic $\pi \rightarrow \pi^*$ transition) and a positive Cotton effect at ca.

345 nm ($n \rightarrow \pi^*$ carbonyl transition). According to the replacement of the 3-phenyl in the isoflavanone structure by a 3-phenoxy substituent in (–)-rotoic acid (**3**), the new compound can be consequently assigned as 3'*R*.

With a slightly more polar chromatographic behaviour and $[\alpha]_D^{23} -80$ (c 0.01, C_5D_5N), compound **6** was also isolated as a white amorphous powder. The HRESMS of the $[M+H]^+$ (found: 427.1414, calcd: 427.1393) evidenced as for **3** the molecular formula $C_{23}H_{22}O_8$. Its UV spectrum λ_{max} (MeOH): 349 nm, clearly differed from that of the previous isomer **3** by a supplementary pronounced shoulder issued from the long wavelength band. Comparison of the 1H and ^{13}C NMR (Table 1) data for both compounds showed that the major difference between them was only cyclization of the C_5H_8 chain exhibiting here one more conjugated double bond causing the above mentioned bathochromic UV shift. The five-membered ring in **3** was replaced by a 2,2-dimethyl pyran likely condensed to 1,2,3-trisubstituted benzoic acid giving rise to a β -tubaic acid moiety.¹⁶ The six-membered ring was characterized in the 1H NMR by four sets of signals divided into two singlets at δ 1.41 and 1.35 ppm, respectively, for two methyls as well as two doublets at δ 6.84 and 5.54 ppm relative to two *cis*-ethylenic H ($J = 10.0$ Hz). The ^{13}C NMR spectrum supported this partial structure by identifying the corresponding C atoms at δ 28.2 and 28.1 ppm for the methyls and δ 130.6 and 118.1 ppm for the ethylenic methines. Finally, the remaining fifth nucleus was quaternary and *O*-bonded and as expected, recorded at δ 77.3 ppm. Furthermore, as observed for (–)-rotoic acid (**3**), the invisible carboxyl proton in C_5D_5N was slightly deshielded to δ 10.95 ppm in $CDCl_3$. On the basis of the above evidence, the new compound **6** was established to be 5-(6,7-dimethoxy-2,3-dihydrochromon-3-oxy) β -tubaic acid. Named (–)-deguoic acid, this metabolite showed a similar CD spectrum to that of (–)-rotoic acid with a negative Cotton effect at ca. 315 nm and a positive Cotton effect at ca. 350 nm also in agreement with the 3'*R*-configuration.

The occurrence of (–)-rotoic acid and (–)-deguoic acid considered as *seco*-rotenoids in *L. nicou* has to be connected to other rare phytochemicals in two other members of the Fabaceae also issued from ring-C opening of either rotenone or deguelin. However, if the newly reported two acids, which are probably the precursors of the corresponding free tubaic acids in *L. nicou*, seem to be closely related to the spiro compounds of *Tephrosia candida*²¹ by the oxidative cleavage of the rotenoid C(12)–C(12a) bond, inversely, they clearly differ from the other ring-C opened group arising from the reductive cleavage of the C(6a)–O(7) rotenoid bond in *Derris trifoliata*.²²

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