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Structure of Isoamericanin A, a Prostaglandin I_2 Inducer, Isolated from the Seeds of *Phytolacca americana* L.

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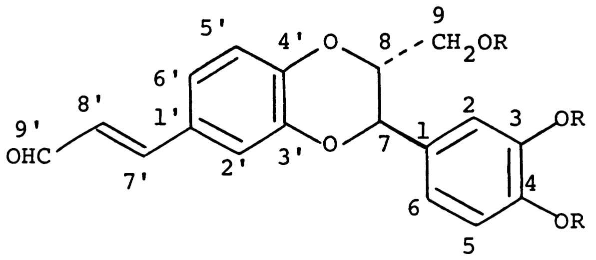
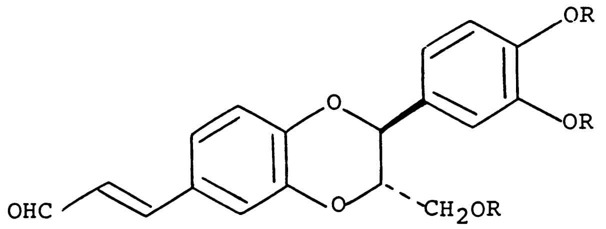
A new *neo*-lignan, isoamericanin A was isolated as a prostaglandin I_2 inducer from the seeds of *Phytolacca americana* L. and its structure was fully characterized by extensive 2D NMR and long range selective proton decoupling (LSPD) techniques.

In the course of our continuing search for prostaglandin I_2 inducers in natural products,[1)] we have isolated a new *neo*-lignan (**1**) named isoamericanin A and racemic americanin A (**2**) previously reported in optically active form by H. Wagner[2)] from a methanol extract of *P. americana*. Although isoamericanin A is likely to be a regioisomer of americanin A[3)] in the substituents on the 1,4-dioxane nucleus, we have met considerable difficulty in distinguishing these two compounds due to the extreme similarity of their spectral properties. Herein we wish to report the full characterization of isoamericanin A.

Compounds (**1**) and (**2**)[4)] were isolated by a combination of silica-gel column, Sephadex LH-20, and HPLC; isoamericanin A, mp 177-178 °C, $[\alpha]_D \pm 0$ (*c* 1.01), m/z 328.0930 (M^+, $C_{18}H_{16}O_6$ requires 328.0947), 166; λ_{max} (EtOH) 218 (ε 26800), 224 (ε 24400), 234 (ε 21100), 250 (ε 16700), 290 (ε 14600), 310 (ε 19500), 330 (ε 19500) nm; ν_{max} (KBr) 3500 (OH), 1630 (C=O), 1600 (conj. C=C), 1580, and 1540 (aroma.) cm^{-1}. All the ^1H NMR signals for **1**, **2**, and their acetates (**1a**) and (**2a**)[5)] could be unambigously assigned by examination of the COSY and NOESY spectra in addition to simple homo-decoupling experiments (Table 1). A ^1H-^{13}C shift correlation 2D NMR spectrum of **1a** led to the straightforward assignments of the ^{13}C NMR signals except for those of the quaternary carbons (δ 128.16, 134.04, 142.59, 142.88, 143.58, 143.65), in addition to three non-substituted aromatic carbons (δ 122.62, 124.01, 125.39), which were correlated to three poorly resolved proton signals at δ 7.26-7.30. The complete assignments of these carbon signals, however, could be made through acquisition of a long-range ^1H-^{13}C correlated 2D NMR spectrum. For instance, 7-H at δ 4.87 was long-range coupled with the carbon signals at δ 134.04, 122.62, and 125.39. The quaternary carbon (δ 134.04) could therefore be assigned to C-1, and the methine carbons at δ 122.62 and 125.39 to C-2 and C-6, respectively. This means that the methine carbon at δ 124.01 which was not coupled with 7-H should be assigned to C-5. However, the two quaternary carbons at δ 142.59 and 142.88 which showed a correlation with the overlapped proton signal region due to 2, 5, and 6-H could not be distinguished to be C-3 or C-4 by this method. This ambiguity could be clarified by comparison with the J_{H-C} values in the undecoupled ^{13}C NMR spectrum. The signal at δ 142.59 having both *ortho*- and *meta*-couplings (3.6 and 6.5 Hz) could be assigned to C-3, whereas that at δ 142.88 showed two kinds of *meta*-coupling (6.5 and 6.9 Hz) and was thus assignable to C-4. Consequently, all the ^{13}C NMR signals of **1a** were identified, and also complete assignment of those of americanin A acetate (**2a**) was carried out by the same method (Table 1).



1: R = H
1a: R = Ac



2: R = H
2a: R = Ac

The problem associated with the regio- and stereochemistries in the substituents at C-7 and C-8 on the 1,4-dioxane ring has remained unsolved. LSPD$^{6)}$ should give a clue to this end as successively applied to a similar problem in the coumarino lignans, cleomiscosin A and B.$^{7)}$ In **1a**, selective irradiation at 7-H (δ 4.98) and 8-H (δ 4.26) led to significant sharpening of the carbon signals for C-3' (δ 143.58) and C-4' (δ 145.65), respectively. In the case of **2a**, the carbon signals for C-4' and C-3', respectively, showed clear sharpening when the signals at 7-H and 8-H were irradiated separately. The *trans* orientation for 7-H and 8-H on the 1,4-dioxane ring was evident from the $J_{7,8}$ value (7.9 Hz). Accordingly, the structure of isoamericanin A must be formulated as **1**, whereas that of americanin A is represented as **2**.

Although americanin A reported by H. Wagner was optically active,

Table 1. ^{13}C and ^{1}H NMR data of **1a**, **2a**

Carbon No.	**1a** Ca)	**1a** Hb)	**2a** Ca)	**2a** Hb)
1	134.04		133.98	
2	122.62	7.26-7.30c)	122.64	7.26-7.30c)
3	142.59		142.60	
4	142.88		142.91	
5	124.01	7.26-7.30c)	124.03	7.26-7.30c)
6	125.39	7.26-7.30c)	125.39	7.26-7.30c)
7	75.80	4.98 (d, 7.9)	76.20	5.02 (d, 7.9)
8	75.73	4.26 (ddd, 7.9, 4.3, 3.7)	75.36	4.24 (ddd, 7.9, 4.0, 3.7)
9	62.27	4.40 (dd, 12.4, 3.7)	62.33	4.40 (dd, 12.4, 3.7)
		4.00 (dd, 12.4, 4.3)		4.01 (dd, 12.4, 4.0)
1'	128.16		128.33	
2'	117.11	7.17 (d, 2.1)	117.04	7.21 (d, 1.8)
3'	143.58		143.17	
4'	145.65		146.06	
5'	117.84	7.01 (d, 8.2)	117.90	6.99 (d, 8.2)
6'	123.01	7.15 (dd, 8.2, 2.1)	122.94	7.13 (dd, 8.2, 1.8)
7'	152.13	7.35 (d, 15.9)	152.13	7.37 (d, 15.9)
8'	127.38	6.57 (dd, 15.9, 7.6)	127.46	6.59 (dd, 15.9, 7.6)
9'	193.44	9.63 (d, 7.6)	193.44	9.65 (d, 7.6)
$Me\underline{C}O_2$	170.25		170.26	
	167.87		167.88	
	167.87		167.86	
$\underline{Me}CO_2$	20.60	2.30 (s)	20.61	2.31 (s)
	20.60	2.295 (s)	20.61	2.30 (s)
	20.60	2.04 (s)	20.56	2.10 (s)

a) 100.61 MHz, in $CDCl_3$, TMS as int. standard.
b) 400 MHz, in $CDCl_3$, TMS as int. standard. Multiplicities and J values (in Hz) are given in parentheses.
c) Poorly resolved signals.

isoamericanin A and americanin A isolated from the same source by us show no specific rotation, indicating that both the compounds must be racemic at C-7 and C-8. The possibility of racemization during the isolation process was excluded by a deutrium experiment. This result supports the biosynthetic hypothesis8) of americanins being formed in the plant *via* a free radical oxidative coupling of

3,4-dihydroxycinnamyl alcohol followed by oxidation of the allylic alcohol, which can give rise to a mixture of regio- and diastereoisomers.

Isoamericanin A (**1**)$^{9)}$ can increase by up to 149.8 % at 10^{-5} M the release of endogenous prostaglandin I_2 (PGI_2) from the rat aorta,$^{10)}$ and also shows a moderate inductive effect on the release of PGI_2 *in vivo*.

References

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3) The structure of americanin A was originally proposed as **1**, but very recently revised to **2**. S. Antus, O. Seligmann, and H. Wagner, Liebigs Ann. Chem., **1986**, 647.
4) **1**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.34 (dd, 12.2, 4.6 Hz, 9-H), 3.55 (dd, 12.2, 2.4 Hz, 9-H), 4.14 (ddd, 7.9, 4.6, 2.4 Hz, 8-H), 4.87 (d, 7.9 Hz, 7-H), 6.71 (dd, 8.2, 1.8 Hz, 6-H), 6.73 (dd, 15.9, 7.6 Hz, 8'-H), 6.77 (d, 8.2 Hz, 5-H), 6.82 (d, 1.8 Hz, 2-H), 7.02 (d, 8.2 Hz, 5'-H), 9.60 (d, 7.6 Hz, 9'-H). **2**: mp 260-262 °C, $[\alpha]_D \pm 0$, m/z 328.0930 (M^+, $C_{18}H_{16}O_6$ requires 328.0947), 166.
5) **1a**: mp 155-156 °C, m/z 454 (M^+), 310, 148; ν_{max} ($CHCl_3$) 1780, 1750, 1680, 1630, 1610, and 1510 cm^{-1}. **2a**: mp 129.5-130.5 °C, m/z 454 (M^+), 310, 148; ν_{max} ($CHCl_3$) 1780, 1750, 1680, 1630, 1615, and 1510 cm^{-1}.
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