

Acetylidene Americanin A, An Artefact Isolated from *Phytolacca americana*

Won Sick Woo, Sam Sik Kang, Otto Seligmann* and Hildebert Wagner*

Natural Products Research Institute, Seoul National University, Seoul 110, Korea and

*Institut für Pharmazeutische Biologie der Universität München, München, W. Germany

(Received 1 June 1982)

Abstract □ From the seeds of *Phytolacca americana*, acetylidene americanin A was isolated and its structure elucidated mainly by spectroscopic methods. This compound is not a genuine constituent but formed in the tissue on heating with acetone.

Keywords □ *Phytolacca americana*, Phytolaccaceae, acetone adduct of americanin A, aldol condensation reaction in plant tissues.

Isolation of a series of new lignans from the seeds of *Phytolacca americana* (Phytolaccaceae) was recently reported.^{1,2)}

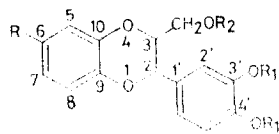
During the extraction of the lignans, it was found that an acetone extract contained a series of compounds having almost the same R_f values as the lignans, but these compounds could be distinguished from the lignans by pink color of their spots on TLC plate sprayed with H₂SO₄ and heated at 110° for 5 min. This paper deals with the isolation and structure elucidation of one of these compounds.

The compound (I), C₂₁H₂₀O₆ (M⁺, 368.1216), crystallized from MeOH as yellowish crystals, mp 242°, [α]_D²⁰ ± 1.0°, showed in its IR the absorption peaks of OH (3400cm⁻¹), α,β-unsaturated CO (1650, 1610cm⁻¹) and an aromatic system (1580, 1510, 1450cm⁻¹). The formation of a di-O-methylether (II), mp 142.5°, a tri-O-acetate (III), mp 144.5° and a di-O-methyl-

ether monoacetate (IV), mp 118-20°, indicated the presence of an alcoholic and two phenolic OH groups. Preparation of a 2,4-DNP derivative, mp 167-70°, confirmed the presence of the carbonyl group.

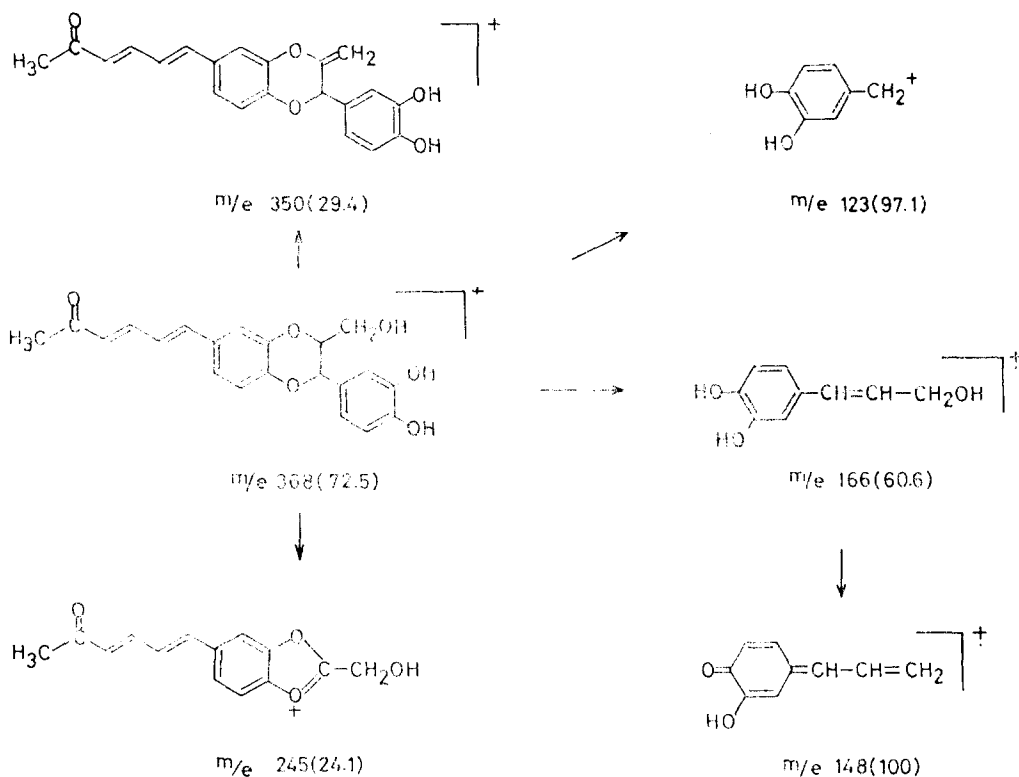
The ¹H-NMR spectrum of I in DMSO-d₆ showed a three proton singlet at 2.25ppm, assignable to methyl protons of an acetyl group, but the compound was resistant to acid treatment suggesting the presence of a methylketone function. It was confirmed by appearance in ¹³C-NMR of I of a methyl carbon signal at δ 27.2(q) and a carbonyl carbon signal at δ 197.9(s) indicating α,β-unsaturated carbonyl function.³⁾

Oxidation of II in cold acetone solution with KMnO₄ resulted in the formation of the 6-carboxyl derivative (V), mp 217°, identical to the oxidation product obtained from di-O-



1'' 2'' 3'' 4''

- | | | |
|-----|----------------------------------|--|
| I | R = CH=CH-CH=CHCOCH ₃ | R ₁ =R ₂ =H |
| II | R = CH=CH-CH=CHCOCH ₃ | R ₁ =CH ₃ R ₂ =H |
| III | R = CH=CH-CH=CHCOCH ₃ | R ₁ =R ₂ =Ac |
| IV | R = CH=CH-CH=CHCOCH ₃ | R ₁ =CH ₃ R ₂ =Ac |
| V | R = COOH | R ₁ =CH ₃ R ₂ =H |
| VI | R = CH=CHCHO | R ₁ =CH ₃ R ₂ =H |



Scheme I: Mass spectral fragmentation of the compound (I).

Figures in parentheses indicate % of base peak.

methylamericanin A (VI) under the same conditions. This result strongly suggested that I was an acetone adduct of americanin A, which structure was recently revised to be trans-2-(3',4'-dihydroxyphenyl)-3-hydroxymethyl 6-(3''-oxo-prop-1''-enyl)-benz[1,4]-dioxane¹⁾.

The mass spectrum of I is in complete agreement with the assigned structure. The principal ion fragments as summarized in Scheme I. Finally, the structure of I was confirmed by the synthesis of II from di-O-methylamericanin A (VI) through the aldol condensation reaction with acetone in the presence of alkali. Therefore the compound (I) has to be regarded as 2-(3',4'-dihydroxyphenyl)-3-hydroxymethyl 6-(5''-oxo-hexa-1'',3''-dien-1''-yl)-benz[1,4]-dioxane.

Several compounds with irregular skeleton possessing acetylidene moiety (C_3 unit) such as ionones⁵⁾ and irones⁶⁾ were found in plants but biogenesis of these compounds has not been fully elucidated. This compound (I) possesses acetylidene moiety, but it is suggested to be an artefact formed by aldol condensation during extraction with acetone, since it was not detected in the extracts prepared using solvents as methanol, dioxane and tetrahydrofuran, which have no methyl ketone group. However, extraction with such solvents of plant material pretreated with acetone gave compound I.

These facts together with failure of adduct formation on heating the methanol extract in acetone and detection of a series of compounds

in a methyl ethyl ketone extract as pink spots on TLC similar to those in the acetone extract clearly indicated that aldol condensation reaction occurred in the seeds.

To our best knowledge, this is the first report on the occurrence of aldol condensation in plant tissues.

EXPERIMENTAL METHODS

Mps were taken on a Mitamura-Riken apparatus and are uncorrected.

Extraction and Isolation of I

The dried seeds were refluxed with acetone. The acetone extract was concentrated, and filtered to remove americanin A. The mother liquor was chromatographed on a SiO₂ column eluting with benzene-ether-MeOH (8 : 2 : 1). I was crystallized from MeOH to yield yellowish crystals, mp 242°, $[\alpha]_D^{20} \pm 0^\circ$ (C=0.55, pyridine) and showed red color with c-H₂SO₄. UV $\lambda_{\max}^{\text{EtOH}}$ nm (log ϵ): 257(4.00), 267(4.00), 285(3.96), 3.49(4.37); IR ν_{\max}^{KBr} cm⁻¹: 3400(OH), 1650, 1610 (α, β -unsaturated CO), 1580, 1510, 1450(aromatic C=C); NMR (DMSO-d₆) δ : 2.25 (3H, s, CH₃CO), 3.50 (2H, m, CH₂O), 4.15(1H, m, H-3), 4.97(1H, d, J=9Hz, H-2), 6.23(1H, d, J=15Hz, H-4''), 6.8~7.2(9H, m, aromatic H and H-1'', 2'', 3''); ¹³C-NMR (DMSO-d₆) δ : 197.9 (CO), 146.0(C-9), 145.4(C-4'), 145.0(C-3'), 144.7(C-10), 144.2, 144.0, 140.9 (C-1'', 2'', 3''), 129.8(C-4''), 129.6(C-6) 127.7(C-1'), 125.6(C-7), 119.1(C-6'), 117.3(C-8), 115.8 (C-5), 115.5(C-5'), 115.2(C-2'), 78.8(C-3), 76.4(C-2), 60.9(CH₂OH), 27.2(CH₃).

Methylation of I

To a sample (I, 100mg) and K₂CO₃(1 g) in dry acetone(100ml) was added dropwise dimethyl sulfate(2ml) with stirring at room tem-

perature. After 3hr, the reaction mixture was filtered, concentrated to a half and poured onto crushed ice. The precipitate was filtered, crystallized from MeOH to afford II as yellowish amorphous powder, mp 142-5°; IR ν_{\max}^{KBr} cm⁻¹: 3500(OH), 3000, 2940, 2850(OCH₃), 1260, 1027(C-O-C).

Acetylation of I

A sample (I, 50mg) was allowed to stand with pyridine and Ac₂O(0.5ml each) at room temperature overnight. The reaction mixture was poured onto crushed ice and filtered. The precipitate was crystallized from CHCl₃-hexane to give III as whitish stouts, mp 144-5°; IR ν_{\max}^{KBr} cm⁻¹: 1770, 1740, 1250, 1200, 1180(acetate); NMR(CDCl₃) δ : 2.04(3H, s, acetyl), 2.31 (9H, s, 2 x acetyl and CH₃CO), 4.25(3H, m, H-3 and CH₂O), 5.04(1H, d, J=8Hz, H-2), 6.22 (1H, d, J=14Hz, H-4''), 6.8-7.4(9H, m, aromatic H and H-1'', 2'', 3'').

Acetylation of II

Di-O-methylether (II, 100mg) was acetylated as described above. Crystallization from acetone-petr. ether gave IV as whitish needles, mp 118-20°; IR ν_{\max}^{KBr} cm⁻¹: 3000, 2940, 2915, 2840(OCH₃), 1740, 1250, 1230(acetate), 1020 (C-O-C); NMR(CDCl₃) δ : 2.05(3H, s, acetyl), 2.27(3H, s, CH₃CO), 3.89(6H, s, 2 x CH₃O), 4.27 (2H, bs, CH₂O), 4.30 (1H, m, H-3), 4.98(1H, d, J=7Hz, H-2), 6.17(1H, d, J=16 Hz, H-1''), 6.7~7.1(9H, m, aromatic H and H-1'', 2'', 3'').

Formation of 2,4-Dinitrophenylhydrazone

To a sample(I, 100mg) in and 2,4-dinitrophenylhydrazine(100mg) in EtOH(150ml) was added 3 drops of c H₂SO₄. The mixture was kept at room temperature for 5 hr and then poured onto crushed ice. The precipitate was filtered, washed with d-H₂SO₄ and then

H₂O. It was crystallized from MeOH to give dark reddish amorphous powder, mp 167-70°, UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm(log ϵ): 259(4.16), 346(4.15), 406(4.42).

Acid Treatment of I

A sample of I(20mg) was refluxed with 5% HCl-MeOH(50ml) for 3hr and treated in the usual way to recover original compound I(TLC and mmp).

KMnO₄ Oxidation of II

To a solution of II(100mg) in acetone(100ml) was added an acetone solution of KMnO₄ dropwise at room temperature until the color of KMnO₄ did not disappear. After degradating an excess of KMnO₄ by addition of EtOH, the reaction mixture was filtered and chromatographed on SiO₂ column eluting with benzene-ether(4 : 1) to yield V. It was crystallized from MeOH as whitish needles(25mg), mp 217°, IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1710(Ar-COOH), the Na salt, IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1610, 1450(COO⁻). It was confirmed by direct comparison with an authentic sample obtained from VI under the same conditions.

Synthesis of II

To a solution of VI(135mg) in MeOH(20 ml) and acetone (2ml) was added 8N-NaOH (2ml). The reaction mixture was allowed to stand at 40° in water bath for 16 hr, added to crushed ice and filtered. The reaction product was chromatographed on a SiO₂ column eluting with benzene-ether (6 : 1) and crystallized from MeOH to give II as yellowish amorphous powder (100mg), mp 142-4°. This proved to be identical by mmp, co-TLC and IR with the authentic sample.

Extraction of the Seeds with Methyl Ethyl Ketone

The dried seeds(50g) were refluxed with methyl ethyl ketone for 3 hr (3 times), filtered

and concentrated in vacuo. The residue was subjected to TLC on silica gel in benzene-ether-MeOH(8 : 2 : 1). A series of compounds appeared as pink spots having almost the same R_f values as lignans by spraying the plate with H₂SO₄ and heating at 110° for 5 min like the acetone extract.

Extraction with Other Solvents

The dried seeds(50g) were refluxed with dioxane, MeOH and THF, separately for 3 hr (3 times), filtered and treated as above. Each TLC chromatogram showed only lignans as bluish green spots without the acetonilidene derivatives. However, when extracted the seeds pretreated with acetone (150g, 3 hr×2 times), the adducts were detected in all the extracts. The MeOH extract was refluxed in acetone (100ml) for 18hr on water bath, concentrated in vacuo and chromatographed on TLC plate; acetonilidene derivatives were not detected.

ACKNOWLEDGMENT

The authors are grateful to KOSEF for financial support.

LITERATURE CITED

- 1) Woo, W.S., Kang, S.S., Wagner, H. and Chari, V.M., Die Struktur von Americanin, einem neuen Neolignan aus *Phytolacca americana*. *Tetrahedron Lett.*, 3239 (1978).
- 2) Woo, W.S., Kang, S.S., Seligmann, O., Chari, V.M. and Wagner, H., The structure of new lignans from the seeds of *Phytolacca americana*. *Tetrahedron Lett.*, 4225 (1980).
- 3) Levy, G.C., Lichter, R.L. and Nelson, G.L., *Carbon 13 nuclear magnetic resonance spectroscopy*, 2nd ed. John Wiley & Sons, N.Y. 1980.
- 4) Szilagi, I., Tetenyi, P., Antus, S., Seligmann,

- O., Chari, V.M., Seitz, M., and Wagner, H., Struktur von Silandrin und Silymonin, zwei neuen Flavanolignanen aus einer weissblühenden *Silybum marianum* Varietät. *Planta Medica*, **43**, 121 (1981).
- 5) Naves, Y.R., Some development in the chemistry of ionones and their derivatives. *J. Soc. Cosmet. Chem.*, **22**, 439 (1971).
- 6) Ruzicka, L., Seidel, C.F. Schimz, H. and Tavel, C., Einige Ergänzende Versuche zur Konstitutionsaufklärung und Synthese des Ions. *Helv. Chim. Acta*, **31**, 257 (1948).