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A CAFFEOYLCYCLOHEXANE-1-CARBOXYLIC ACID DERIVATIVE FROM *ASIMINA TRILOBA*

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Key Word Index—*Asimina triloba*; Annonaceae; *Eurytides marcellus*; Papilionidae; Graphiini; caffeoylcyclohexane-1-carboxylic acid isomers; 3-caffeoyl-*muco*-quinic acid; chlorogenic acid; oviposition stimulants.

Abstract—Three caffeoyl derivatives of 1,3,4,5-tetrahydroxycyclohexane 1-carboxylic acid, one of them an oviposition stimulant for the zebra swallowtail butterfly, *Eurytides marcellus*, were isolated from *Asimina triloba*. The structure of the active isomer, 3-caffeoyl-*muco*-quinic acid, was determined by extensive NMR studies. © 1998 Published by Elsevier Science Ltd. All rights reserved

INTRODUCTION

In the course of research on the oviposition stimulants of swallowtail butterflies of the tribe Papilionini (Family: Papilionidae), we and others have found chlorogenic acid and related compounds to be important components of the chemical profiles by which the insects recognize their host plants. In particular, 5-caffeoylquinic acid (5-CQA, *trans*-chlorogenic acid) is a component of the stimulatory profiles for *Papilio polyxenes* from *Daucus carota* (Apiaceae) [1], and *P. protenor* from *Fagara ailanthoides* (Rutaceae) [2].

In recent studies of the oviposition behavior of the zebra swallowtail, *Eurytides marcellus* (tribe-Graphiini) [3], which exclusively attacks plants of the genus *Asimina* (Annonaceae), we isolated three caffeoyl derivatives from *Asimina triloba*, one of which (compound **1**) was found to stimulate oviposition by the female butterflies [Haribal and Feeny unpublished]. Here we describe the structure of **1**, which was found to be a caffeoyl derivative of 1,3,4,5, tetrahydroxycyclohexane 1-carboxylic acid and an isomer of 5-CQA. Since the configuration of CQA isomers is of significant biological interest, and since there is confusion in the literature as to the nomenclature of their structures, we have used extensive NMR experiments to determine the structure of this compound.

RESULTS

Behavioral bioassays revealed that an ethanolic extract of *A. triloba* was active in stimulating oviposition by *E. marcellus* females. On fractionation into chloroform (A) and post-chloroform aq. (B) fractions, the major oviposition activity was found to be in fraction B. Fraction B was further separated into four fractions, B1–B4, by means of HPLC. Most of the activity was recovered in fraction B2. Compounds **1–3** were isolated from fraction B2 by HPLC. Only compound **1** was found to be an oviposition stimulant for the butterflies. Compounds **1–3** were all isomers of caffeoyl derivatives, as shown by their typical UV spectral absorption, and shared the same molecular weight.

Identification of Compound **1**

Compound **1** had an UV absorption at λ_{max} 225 and 325 nm in MeOH. The FAB mass spectrum gave an $M^+ + 1$ at 355, giving a MF of $C_{15}H_{18}O_9$ and indicating **1** to be a derivative of caffeoylquinic acid. In ^{1}H NMR the peaks at δ 7.45 (1H, *d* J = 15 Hz), 7.01, (1H, *d* J = 1.6 Hz), 6.96 (1H, *dd*, 1.6 and 8.0 Hz), 6.75 (1H, *d* J = 8 Hz), 6.19 (1H, *d* J = 15 Hz) (Table 1) confirmed the presence of a caffeoyl moiety and the J value of 15 Hz for the allylic protons (C-7′ and C-8′) indicated it to be a *trans* isomer. 5-CQA is the most commonly reported isomer from plants. However, the HPLC retention time of commercially available 5-CQA was found to be greater than that of **1** and its ^{1}H NMR spectrum also differed in the quinic acid region. On the basis of the geHMQC (gradient enhanced) [4] spectrum of compound **1**, the multiplets at δ 5.17, 3.52 and 3.86, attached to the carbon signals at δ 70.9, 72.8, 67.2 respectively (Table 1), were

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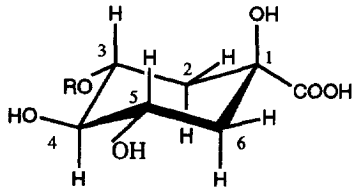

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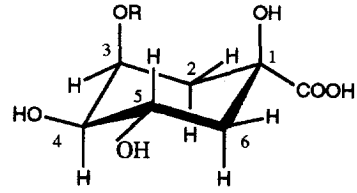

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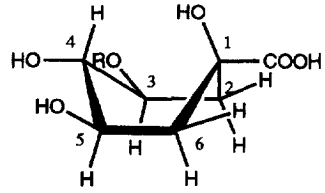

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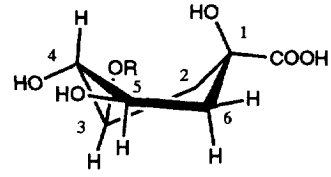

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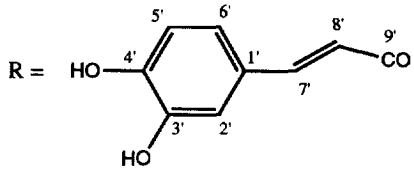

1. 3-caffeoyl-*muco*-quinic acid
4. 3-caffeoylquinic acid

Table 1. 1D and 2D NMR data of compound **1** (δ in ppm, J in Hz and solvent DMSO-d_6)

Carbon	^{13}C NMR	^{1}H NMR	J values	DQCOSY	HMBC
1	79.1				
2	38.6	1.80 (a), 2.58 (b)	2 *dd*, 13.1 ($^{2}J_{2ab}$), 7.5 ($^{3}J_{2a3}$), 4.6 ($^{3}J_{2b3}$)	2.58, 5.17 1.8, 5.17	36.5 (C-6), 70.9 (C-5), 175.9 (C-7)
3	70.9	5.17	*ddd*, 9.2 ($^{3}J_{34}$)	1.8, 1.9, 3.52	38.6 (C-2), 166.0 (C-9) (weak)
4	72.8	3.52	*m*	5.17, 3.86, 4.86	
5	67.2	3.86	*ddd*, 6.1 ($^{3}J_{45}$)	3.52, 1.8, 1.9	70.9 (C-3), 36.5 (C-6), 38.6 (C-2)
6	36.5	1.89 (a) 1.98 (b)	2 *dd*, 13.6 ($^{2}J_{6ab}$), 5.7 ($^{3}J_{6a5}$), 4.2 ($^{3}J_{6b5}$)	1.98, 3.86 1.89, 3.86	38.6 (C-2), 72.8 (C-4), 175.9 (C-7) 67.2 (C-5), 72.8 (C-4), 175.9 (C-7)
7	175.9				
1′	125.6				
2′	115.7	7.01	*d*, 1.6	6.96	
3′	148.1				
4′	145.5				
5′	114.9	6.75	*d*, 8.0	7.01	
6′	121.0	6.96	*dd*, 8.0, 1.6	6.75, 7.01	
7′	115.7	6.19	*d*, 15.0	7.45	
8′	144.3	7.45	*d*, 15.0	6.19	121.0 (C-6′), 115.7 (C-2′)
9′	166.0				114.9 (C-5′), 115.7 (C-2′), 70.9 (C-3)
1-OH		4.86	*d*, 3.6	3.52	70.9 (C-3), 67.2 (C-5), 36.5 (C-6), 38.9 (C-2)
4-OH		4.10 or 4.70	*br*		
5-OH		4.10 or 4.70	*br*		
7-OH		12.12 (OH)	*br*		
3′-OH		9.52	*s*		145.5 (C-4′), 115.7 (C-2)
4′-OH		9.14	*s*		148.1 (C-3), 114.9 (C-6)

Table 2. *J* values (in Hz) observed and simulated

Compound	Solvent	$(^2J_{2a2b})$	$(^2J_{6a6b})$	$(^3J_{2a3})$	$(^3J_{2b3})$	$(^3J_{34})$	$(^3J_{45})$	$(^3J_{56a})$	$(^3J_{56b})$	References
1	DMSO-d_6 1D					12.5	6.5			
	E-COSY(*R*)	13.1	13.6	7.5	4.6	9.2	8.7	5.7	4.2	
	Simulated					7.3	6.1	6.4	4.6	
syello-QA	NaoD–D_2O			8	6	9	9	9	6	[8]
epi-QA	NaoD–D_2O			6	4	3	6.5	8	5	[8]
Chamigrenes*				13,0 (8α, 7β)	1.8 (8α, 7β)	5.8 (10α, 11β)	2.2 (10α, 11α)	13 (10β, 11α)	2.2 (10β, 11β)	[11]

* Chamigrene (TBα-10 as reported in the paper).

assigned to the three -CHOH- moieties of quinic acid. The downfield shift of one of the non-hydroxyl protons of -CHOH- (δ 5.17) groups suggested that the caffeoyl group is attached at either C-3 or C-4 of quinic acid. In the geHMBC spectrum, C-9′ showed a weak correlation to C-3, confirming that the substitution is at C-3. Since all quinic acid protons showed complex multiplicity, and since we could not obtain coupling constants with 1D ^{1}H NMR, it was difficult to assign stereochemistry to the quinic acid moiety. ECOSY [4–7] and DQCOSY experiments [8], which have been used in assigning the stereochemistry of sugar protons, were therefore used to calculate the coupling constants for all the protons involved (Tables 1 and 2). The high coupling constant values for $^3J_{2a3}$, $^3J_{2b3}$ (7.5 and 4.6 Hz) and $^3J_{34}$ (9.2 Hz) revealed that the protons at 3 and 4 are axial. Similarly, values for $^3J_{45}$ (8.7 Hz) and $^3J_{56a}$, $^3J_{56b}$ (5.7 and 4.2 Hz) showed that the proton at C-5 is also axial in orientation. The *J* values of substituted cyclohexanes have been shown to be in the range of ~10 Hz for axial-axial and ~5 Hz for axial-equatorial if the substitution is equatorial and in the order of ~2–3 Hz for both axial-equatorial and equatorial-equatorial protons if the substituents are axial [9]. The *J* values are slightly lower than would be expected for a perfect chair conformation, suggesting that the quinic acid moiety of **1** may exist in a skewed or boat form. These *J* values were further compared with simulated spectra, whose pattern closely matched the above results (Table 2). On the basis of these values and comparison of reported values for other quinic acid derivatives we concluded that all non-hydroxyl protons of the -CHOH- groups were axial in orientation. These values are also comparable to those reported for *scyllo*-quinic acid (Table 2) by Corse and Lundin [10].

The configuration at C-1 could not be decided by the above methods. Of the 6 hydroxyl protons of **1**, peaks at δ 9.14 and 9.52 were assigned to the phenolic hydroxy groups attached to C-3′ and C-4′, respectively. The broad peak of δ 12.12 was assigned to the hydroxyl proton of the carboxylic acid at C-1. A doublet at δ 4.86, and broad peaks at 4.7 and 4.10 were assigned to the alcoholic protons of the quinic acid moiety. In the DQCOSY spectrum the peak at δ 4.86 was correlated with the peak at δ 3.52 (C-4), the coupling constant being 3.6 Hz. It seems, therefore, that this C-4 proton is either coupled with the C-4 hydroxy proton or is bonded by through-space coupling [$^6J_{(HH)}$ type] to the C-1 hydroxy proton. Carmely *et al.* (1986) have shown similar couplings for swinholides [11]. Such coupling is possible only if the orientation of the C-1 hydroxy group is axial and the cyclohexane is either in the boat form or a skewed chair form. The *J* values observed by Guella *et al.* (1992) for protons of α-chamigrene derivatives containing a cyclohexane ring in the boat form also support our observations (Table 2) [12, 13].

On the basis of this evidence, as well as comparison with literature values [14–22], we conclude that **1** is 1*S*-3β-*O*-*trans*-caffeoyl-1α,4α,5β-trihydroxycyclohexane-1-carboxylic acid and that the molecule probably exists in a boat form or skewed chair form. Compound **1** is thus a previously unreported 3-*O*-caffeoyl derivative of *muco*-quinic acid (3-C*m*QA), in which the substituted 3-hydroxy group is equatorial. The isomeric 3-caffeoylquinic acid (3-CQA), compound **4**, in which the 3-hydroxy group is axial, has been reported from coffee, artichoke and other plants [17, 23–25]. Of the ^{13}C NMR values calculated for various isomers [26] and derivatives of tetrahydroxycyclohexane 1-carboxylic acid, those of 1*S*-3β-*O*-acetyl-1α,4α,5β-trihydroxycyclohexane 1-carboxylic acid matched our observed values most closely (Table 3). Though our evidence points to an axial orientation for the C-1 hydroxy, unambiguous confirmation of the absolute stereochemistry of **1** must await further research, perhaps including X-ray crystallographic studies.

The structures of compounds **2** and **3** are not yet fully characterized, but the work is in progress.

DISCUSSION

Many derivatives of hydroxycinnamic (caffeoyl, feruloyl, and sinapyl) acids and their esters of quinic

Table 3. Comparison of ^{13}C NMR values for various 1,3,4,5-tetrahydroxycyclohexane-1-carboxylic acid derivatives and isomers (calculated and observed)

Cyclohexane carbons	Steric orientation	R = H	R = Ac	R = *trans*-caffeoyl (B2212)*	R = *trans*-caffeoyl (CQA-2)*	
1	OH(a), COOH(e)	78.4	79.4	*79.1*	*79*	
2	H(a), H(e)	39.3	40.3	*38.6*	*38.9*	
3	OR(e), H(a)	65.5	68.5	*70.9*	*68.2*	
4	OH(e), H(a)	85.3	75.3	*72.8*	*75.0*	
5	OH(e), H(a)	65.5	66.5	*67.2*	*67.0*	
6	H(a), H(e)	39.3	39.3	*35.0*	*34.8*	
		R = R′ = H	R = Ac; R′ = H	R = R′ = H quinic acid*	R = H R′ = Ac	R = H; R′ = Caffeoyl (5-CQA)†
1	OH(a), COOH(e)	74.4	75.4	*74.0*	75.4	*73.3*
2	H(a), H(e)	36.3	34.3	*37.2*	37.3	*38.7*
3	OR(a), H(e)	61.5	64.5	*66.5*	62.5	*71.2*
4	OH(e), H(a)	82.3	80.3	*74.3*	79.3	*76.0*
5	OR1(e), H(a)	61.5	62.5	*68.7*	60.5	*71.9*
6	H(a), H(e)	36.3	39.3	*40.3*	33.3	*38.1*
		R = H	R = Ac			
1	OH(e) COOH(a)	82.4	85.4			
2	H(a), H(e)	41.3	38.3			
3	OR(e), H(a)	69.5	72.5			
4	OH(e), H(a)	85.3	82.3			
5	OH(e), H(a)	69.5	70.5			
6	H(a), H(e)	41.3	41.3			
1	OH(e) COOH(a)	78.4				
2	H(a), H(e)	35.3	33.0			
3	OH(a), H(a)	61.5				
4	OH(e), H(a)	82.3				
5	OH(e), H(a)	69.5				
6	H(a), H(e)	39.3	36.2			

* Literature values or observed values (in italics) in DMSO-d_6.

† Observed values in MeOH-d_4.

e = equatorial; a = axial.

Note: Since reported values for -COOH substitution in cyclohexane were not available, corresponding straight chain values were used.

acid occur commonly in plants. The compounds have various biological effects, including action as antioxidants, growth regulation, antifungal activity and modification of the feeding and oviposition behavior of herbivorous insects [27–33].

Among the swallowtail butterflies (family Papilionidae) there are now reports of caffeoylquinic acids or related compounds acting as oviposition stimulants for species in three of the most recently derived tribes, Papilionini, Troidini and Graphiini [3]. 5-CQA in the foliage of carrot, *Daucus carota*, and parsnip, *Pastinaca sativa*, acts as a contact stimulant for females of *Papilio polyxenes* (tribe Papilionini), which oviposit naturally on these and many other species in the Apiaceae [1; and M. Carter, K. Sachdev-Gupta and P. Feeny, unpublished results]. The compound is present on the leaf surfaces of carrot, where it is detected by contact chemoreceptors on the tarsi of females during drumming behavior. [34, 35]. 5-CQA in the foliage of *Fagara ailanthoides* is also an oviposition stimulant for females of *P. protenor*, another papilionine swallowtail [2]. Quinic acid contributes to the chemical profile that stimulates oviposition by *P. protenor* on *Citrus unshiu*, another of its host plants in the Rut-

aceae [2]. Caffeoylquinic acid derivatives have not been reported as oviposition stimulants for swallowtails in the sister tribe Troidini, whose species feed on the Aristolochiaceae. However, 3-hydroxy-4-methoxycinnamoyl malic acid (3-feruloyl malic acid) is an oviposition stimulant, along with aristolochic acids and sequoyitol, for *Parides alcinous* [36].

Chlorogenic acid compounds have been of interest to chemists for several decades. Quinic acid is produced biosynthetically from the shikimic acid pathway [37]. D-(−)-quinic acid is one of eight possible diastereomers of 1,3,4,5-tetrahydroxycyclohexane-1-carboxylic acid, which has four asymmetric centers. Further substitution of the caffeoyl group, which can exist in either the *cis* or *trans* form, can produce as many as 56 monocaffeoyl isomers, several of which also exist as optical isomers. Due to biosynthetic constraints and the different stabilities of possible isomers, however, the number of isomers found in nature is likely to be considerably smaller than the theoretical maximum. Because of their highly polar nature and sensitivity to pH changes, it has been difficult to separate naturally occurring isomers in the past. Nevertheless, about 16 isomers have been reported and at least partially characterized so far. 5-CQA is the most common of these. Most of the other isomers have been reported from coffee beans, because of the important role played by chlorogenic acids in its flavor [38].

The literature on caffeoylquinic acid derivatives is in a state of some confusion. Although IUPAC established a procedure in 1972 for the naming of these derivatives, the old nomenclature continues to be used by many investigators so that is often hard to distinguish which isomer is being described. Also, comparison of published ^{1}H NMR values is made more difficult by lack of consistency in the choice of solvents. the chemical shifts of the non-hydroxyl protons of the -CHOH- groups of quinic acid are shifted downfield by as much as 1–2.00 ppm in pyridine-d_5 relative to the corresponding values in D_2O and MeOH-d_4. In the last two solvents, furthermore, valuable information on hydrogen and long-range coupling, and hence on stereochemistry, may be lost due to deuterium exchange. The only detailed stereochemical structure of a naturally occurring CQA derivative that we could find in the literature was that of 5-CQA [22]. In the present work, improved techniques such as ECOSY, geHMBC, DQCOSY and geHMQC have allowed us to calculate and assign the coupling constants involved with each proton of quinic acid and also its correlation with the carbon skeleton of the molecule.

5-CQA is not active as an oviposition stimulant for *E. marcellus* [Haribal and Feeny, unpublished results] and is absent from extracts of *Asimina* species. Although it seems surprising that *E. marcellus* females would rely so much for host recognition on a CQA derivative, it is possible that the CQA profile of *Asimina* species, with its dominance by **1**, is sufficiently unique to provide an adequate basis for accurate plant recognition.

EXPERIMENTAL

General

All NMR data were recorded in solvent DMSO-d_6 at 400 or 500 MHz; HPLC was performed on a C18 rp column (IB SIL 5 250 × 10.0 mm. 5 μ, Phenomenex) eluted with 1% HOAc in water and increasing methanol gradient, using a Waters 490 HPLC system and a photodiode array detector (PDA, Millipore) for peak recognition. LC-MS mass spectra were recorded as positive ion electro-spray spectra using a Quatro 1, Micromass instrument.

Plants

Asimina triloba for extraction was collected from Selinsgrove, PA, and Ashantee, near Rochester, NY, U.S.A.

Extraction of hydroxycinnamic acid derivatives

Fresh young leaves of *A. triloba* were extracted with boiling 95% EtOH (4 times the weight of plants) for about 5 min and blended in a Waring blender. Insoluble material was filtered off. The extract was concentrated under red. pres. and partitioned repeatedly between $CHCl_3$ and water until no color was extracted into the $CHCl_3$ layer. The aq. layer was further sepd by HPLC and four frs B1–B4 were collected. Fraction B2 contained several caffeoyl derivatives as determined by the UV absorption of the peaks. these were purified by repeated HPLC.

Compound **1**. Powder; UV (MeOH) λ_{max} at 225 and 325 nm; FABMS *m/z*: 355 [$M^+ + 1$]; MW $C_{15}H_{18}O_9$; NMR (Table 1).

Compound **2**. Powder; UV (MeOH) λ_{max} at 225 and 317 nm; LC-MS: *m/z*: 335 [$M^+ + 1$].

Compound **3**. Powder; UV (MeOH) λ_{max} at 225 and 325 nm; LC-MS: *m/z*: 355 [$M^+ + 1$].

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