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CUCURBIC ACID AND ITS 6,7-STEREOISOMERS

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Key Word Index—*Anemia phyllitidis*; Schizaeaceae; *Juglans regia*; Juglandaceae; *Secale cereale*; Poaceae; jasmonic acid; cucurbic acid.

Abstract—The derivatives 7-*iso*-cucurbic acid (7-*iso*-CA) and 6-*epi*-7-*iso*-cucurbic acid (6-*epi*-7-*iso*-CA) were prepared from (−)-jasmonic acid (JA) by reduction and cucurbic acid (CA) and 6-*epi*-cucurbic acid (6-*epi*-CA) from (+)-7-*iso*-jasmonic acid (7-*iso*-JA). The chromatographic properties (TLC, HPLC, GC) of these derivatives are described and the structures established by physical data. The extracts of different plant materials were analysed with respect to the natural occurrence of JA and CA and its 6,7-stereoisomers. JA, 6-*epi*-CA and 6-*epi*-7-*iso*-CA were identified in female flowers of *Juglans regia* and JA, CA, 6-*epi*-CA and 6-*epi*-7-*iso*-CA in spores of *Anemia phyllitidis*. JA, CA, 6-*epi*-CA and 9, 10-dihydro-JA were detected in immature caryopses of *Secale cereale*. In rye JA dominated in young caryopses, while CA level exceeded the amount of JA in the premature fruits. In each tissue 6-*epi*-CA was found to be a minor component, while CA or 6-*epi*-7-*iso*-CA represented the major components; 7-*iso*-CA could not be detected.

INTRODUCTION

Since the discovery of (−)-jasmonic acid (JA, **1**) and its methyl ester as native compounds in *Vicia faba* and *Artemisia absinthium* respectively [1, 2] and the proof of their widespread occurrence within the plant kingdom [3], JA has attracted the attention of plant physiologists. In addition to JA, its isomer (+)-7-*iso*-jasmonic acid (7-*iso*-JA, **2**) is also known to occur naturally and to show similar biological activities [4, 5]. The natural occurrence of many JA related compounds and a series of conjugates of amino acids have been described and the main pathways of biosynthesis and metabolism have been reported [6–8, and refs therein].

Among the closely related compounds which can be derived from either JA or 7-*iso*-JA by reduction of the keto group at C-6 (Fig. 1), we know only cucurbic acid (CA, **6**) and 6-*epi*-7-*iso*-cucurbic acid (6-*epi*-7-*iso*-CA, **3**) as natural products [9–12], while two further possible 6,7-stereoisomers of CA, 7-*iso*-cucurbic acid (7-*iso*-CA, **4**) and 6-*epi*-cucurbic acid (6-*epi*-CA, **5**), have not previously been considered. Complete physicochemical data have been reported only for CA [10], while for 6-*epi*-7-*iso*-CA only the mass spectrum has been published [11, 12]. We report the synthesis of CA and its 6,7-stereoisomers, their chromatographic and physical data, as well as their qualitative natural occurrence in different plant materials.

RESULTS

CA and its 6,7-stereoisomers (Fig. 1) were synthesized by $NaBH_4$ reduction of JA and 7-*iso*-JA, obtained from cultures of *Botryodiplodia theobromae*, and purified by column chromatography, TLC and HPLC. The R_f-values on TLC in system 2 (free acids) were JA/7-*iso*-JA (0.70), 6-*epi*-CA (0.64), 7-*iso*-CA (0.59) 6-*epi*-7-*iso*-CA (0.52) and CA (0.45). A clear separation of the isomers was achieved by HPLC of the methyl esters, while as free acids JA and 6-*epi*-CA were incompletely resolved (Table 1). The HPLC purified substances were used for the establishment of their physical properties.

Optical rotation of both free acids and methyl esters of JA derivatives are given in Table 2. Both derivatives of JA exhibit a positive $[\alpha]_D$ value while JA shows a negative value (−83.5° [13]; −81.7° [2]). The $[\alpha]_D$ value of the 7-*iso*-JA derivatives (Table 2) is decreased compared to the original compound (+64° [14]).

6-*epi*-7-*iso*-CA-Me and 6-*epi*-CA-Me can be separated from each other as well as from CA-Me and 7-*iso*-CA-Me by GC. The R_ts of the latter two compounds are close (Table 3). However, the mass spectra of CA-Me and 7-*iso*-CA-Me differ significantly (Table 4). In 7-*iso*-CA-Me and 6-*epi*-7-*iso*-CA-Me a McLafferty rearrangement involving the tertiary bonded hydrogen at C-7 forms the complementary ions at *m/z* 152 [type (**a** − H)] and 74 (**b**) (Fig. 2). In CA-Me the ion **a** at *m/z* 153 originating by a simple bond cleavage at C-3 dominates over (**a** − H) at *m/z* 152. The ion at *m/z* 152 represents the precursor ion of the very abundant key ion at *m/z* 134 (**a** − H − H_2O). Another clear difference is the appearance of an intense

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Abbreviations: JA [=(3*R*,7*R*)JA], jasmonic acid; 7-*iso*-JA [=(3*R*,7*S*)JA], 7-*iso*-jasmonic acid; CA, [=(3*R*,6*S*,7*S*)CA], cucurbic acid; 6-*epi*-CA, [=(3*R*,6*R*,7*S*)CA], 6-*epi*-cucurbic acid; 7-*iso*-CA, [=(3*R*,6*S*,7*R*)CA], 7-*iso*-cucurbic acid; 6-*epi*-7-*iso*-CA [=(3*R*,6*R*,7*R*)CA], 6-*epi*-7-*iso*-cucurbic acid.

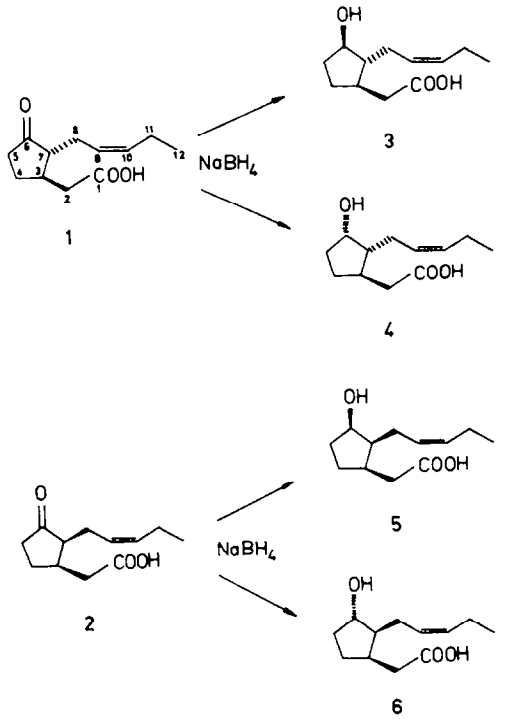

Fig. 1. Structures of JA (1) and 7-*iso*-JA (2) as well as JA derivatives 3 (6-*epi*-7-*iso*-CA), 4 (7-*iso*-CA), 5 (6-*epi*-CA) and 6 (CA) derived by reduction of the keto group.

ion at *m/z* 83 (100%) in CA-Me. Its formation is explained by a McLafferty type rearrangement of ion **a** (*m/z* 153) resulting in the loss of the side chain at C-7. The same is true for the formation of ion **c** (*m/z* 156, $[M-C_5H_{10}]^+$). This process induced by the double bond between C-9 and C-10 is only favoured in CA-Me due to the *cis* arrangement of the proton at C-6 and the pentenyl side chain at C-7 (Fig. 2). Thus, all four JA derivatives can be clearly distinguished by their GC-MS data.

Table 1. HPLC of JA and JA derivatives in MeOH–HOAc (0.2%) (1:1) for free acids or MeOH–H_2O (11:9) for methyl esters (see Experimental)

	R_t (min)	
	Free acid	Methyl ester
JA	22.7	16.6
6-*epi*-CA	22.5	23.0
7-*iso*-CA	20.5	20.3
6-*epi*-7-*iso*-CA	19.5	17.9
CA	17.3	19.5

The 250 MHz proton NMR spectra of CA and its 6,7-stereoisomers were found to differ in chemical shifts and coupling constants of the assigned signals (Table 5). Although the assignments of signals were supported by two dimensional homonuclear shift correlated proton spectroscopy (COSY), not all signals could be resolved (even at 250 MHz).

All the signals show significant variations in chemical shift depending on the stereochemistry at C-6 and C-7 with the exception of the C-12 methyl and C-1 methoxy groups. The most remarkable difference in chemical shift was measured in the case of the single proton at C-3 with a difference of *ca* 1 ppm between its resonance in CA-Me and 6-*epi*-7-*iso*-CA-Me. A *cis* arrangement of the proton at C-3 and the hydroxyl group at C-6 causes a downfield shift of *ca* 0.4 ppm in comparison to the *trans* arrangement. On the other hand, a *cis* arrangement of the proton

Table 2. Optical rotation $[\alpha]_D$ of the JA derivatives as free acids and methyl esters

	Free acid			Methyl ester		
	c (g/100 ml)	*T* (°)	$[\alpha]_D$	*c* (g/100 ml)	*T* (°)	$[\alpha]_D$
6-*epi*-CA	0.10	24.2	+6.4°	0.48	23.5	+10.7°
7-*iso*-CA	0.50	25.3	+20.8°	0.5	22.5	+64.2°
6-*epi*-7-*iso*-CA	0.50	24.5	+4.5°	0.5	22.2	+43.1°
CA	0.40	25.3	+9.6°	0.5	22.5	+14.0°
CA [10]	0.32	26.0	+25.0°			

Table 3. R_t and Kovats retention index (KRI) of JA derivatives by GC analysis

	System 1		System 2	
Compound	R_t (min)	KRI	R_t (min)	RR_t (JA-Me)
JA-Me	9.11	1607	12.72	1.000
6-*epi*-7-*iso*-CA-Me	9.48	1625	12.91	1.015
6-*epi*-CA-Me	9.67	1634	13.08	1.028
CA-Me	10.21	1661	13.44	1.057
7-*iso*-CA-Me	10.26	1663	13.51	1.062

Table 4. GC-MS data [*m/z* (rel. int.), R_t system 1] of synthetic CA and its isomers compared with native compounds isolated from different plant sources

	6-*epi*-7-*iso*-CA-Me			7-*iso*-CA-Me	6-*epi*-CA-Me			CA-Me	
m/z	Synthetic	*J. regia*	*A. phyllitidis*	Synthetic	Synthetic	*J. regia*	*A. phyllitidis*	Synthetic	*A. phyllitidis*
226 $[M]^+$	(2)	(2)	(2)	(2)	(3)	(3)	(3)	(1)	(2)
208 $[M-H_2O]^+$	(12)	(14)	(14)	(10)	(12)	(12)	(11)	(9)	(7)
195 $[M-OMe]^+$	(0)	(0)	(0)	(14)	(16)	(14)	(13)	(2)	(1)
165	(7)	(8)	(9)	(14)	(17)	(15)	(15)	(7)	(6)
156 **c**	(0)	(0)	(0)	(2)	(2)	(1)	(1)	(13)	(11)
153 **a**	(7)	(8)	(13)	(30)	(33)	(31)	(31)	(73)	(72)
152 (**a** − H)	(19)	(19)	(31)	(57)	(63)	(57)	(59)	(32)	(31)
139	(24)	(28)	(32)	(38)	(42)	(37)	(37)	(11)	(11)
134	(100)	(100)	(100)	(96)	(96)	(95)	(96)	(33)	(30)
119	(30)	(31)	(36)	(60)	(61)	(54)	(58)	(16)	(16)
83	(38)	(35)	(36)	(46)	(48)	(44)	(46)	(100)	(100)
79	(59)	(55)	(57)	(100)	(100)	(100)	(100)	(66)	(64)
74 **b**	(22)	(24)	(23)	(28)	(30)	(27)	(29)	(26)	(24)
R_t (min)	9.479	9.639	9.513	10.260	9.661	9.816	9.735	10.205	10.432

Table 5. ^1H NMR data of the methyl esters of the four CA isomers (250 MHz, pyridine-d_5, TMS)

H	6-*epi*-7-*iso*-CA-Me	7-*iso*-CA-Me	6-*epi*-CA-Me	CA-Me
2a	2.75 *dd* (2a/2b = −15.1; 2a/3 = 5.3)	2.72 *dd* (2a/2b = −14.7; 2a/3 = 4.3)	2.85 *dd* (2a/2b = −15.4; 2a/3 = 10.8)	2.56 *dd* (2a/2b = −15.2; 2a/3 = 6.4)
2b	2.46 *dd* (2b/3 = 9.1)	2.30 *dd* (2b/3 = 9.6)	2.77 *dd* (2b/3 = 4.7)	2.38 *dd* (2b/3 = 9.4)
3	2.12 *m*	2.54 *m*	*ca* 2,7* *m*	3.06 *m*
6	4.17 *m*	4.44 *m*	4.39 *m*	4.32 *m*
9	5.48 *m* (9/10 = 10.8)	5.49 *m* (9/10 = 10.8)	5.46 *m* (9/10 = 10.8)	5.44 *m* (9/10 = 10.9)
10	5.60 *m* (10/11 = 7.5)	5.70 *m* (10/11 = 7.5)	5.62 *m* (10/11 = 7.5)	5.51 *m* (10/11 = 7.5)
H_2-11	2.08 *br quin* (11/12 = 7.5)	2.16 *br quin* (11/12 = 7.5)	2.13 *br quin* (11/12 = 7.5)	2.04 *br quin* (11/12 = 7.5)
H_3-12	0.93 *t*	0.95 *t*	0.94 *t*	0.91 *t*
OMe	3.64 *s*	3.65 *s*	3.65 *s*	3.64 *s*

*Overlapped.

Coupling constants (*J*) in Hz in parentheses.

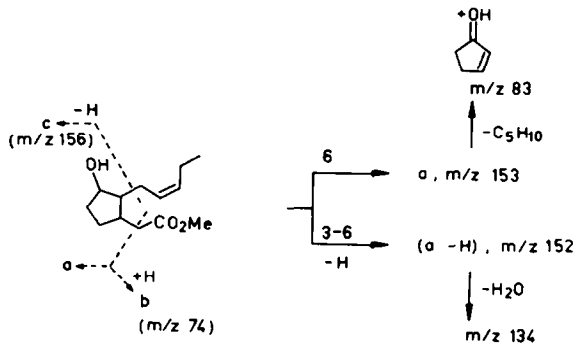

Fig. 2. Fragmentation pattern of 6,7-stereoisomers of CA-Me.

at C-3 and the pentenyl side chain at C-7 results in a high field shift of *ca* 0.5 ppm. This effect should be related to shielding by the C–C bond between C-7 and C-8. Similarly, the single proton at C-6 is shielded by *cis* arrangement to the pentenyl side chain at C-7 and deshielded by *cis* arrangement to the acetyl side chain at C-3. Thus, the signal of the proton at C-6 exhibits its highest shift in 7-*iso*-CA-Me and its lowest shift in 6-*epi*-7-*iso*-CA-Me. The vicinal coupling constant between the two olefinic protons at C-9 and C-10, which was measured to be *ca* 11 Hz, agrees with the *cis* arrangement of both protons.

Natural occurrence of JA, CA and its 6,7-stereoisomers

Juglans regia. The ethyl acetate extract of female walnut flowers before bloom were extracted and purified by DEAE-Sephadex A-25 and TLC (system 2) to give a JA corresponding fraction (R_f 0.50–0.63) and a more polar one (R_f 0.36–0.49). Both fractions were methylated and then analysed by GC-MS (system 1). The JA fraction contained a compound with R_t 9.122 min and a mass spectrum identical with authentic JA-Me [2]. In the more polar fraction, 6-*epi*-7-*iso*-CA and 6-*epi*-CA were detected and identified (Table 4). On the basis of GC-peak area, the predominant isomer was 6-*epi*-7-*iso*-CA-Me, while 6-*epi*-CA-Me was the minor component. This is the first time that the endogenous occurrence of 6-*epi*-CA has been reported.

Anemia phyllitidis. Spores of *A. phyllitidis* were extracted and purified on DEAE-Sephadex A-25, and the JA/CA fraction (0.25 M HOAc in 80% MeOH) was methylated and investigated by GC-MS (system 1). The predominant JA derivative was found to be CA-Me, while 6-*epi*-CA-Me and 6-*epi*-7-*iso*-CA-Me gave smaller peak areas of 22 and 3%, respectively (CA-Me = 100%). The identification of CA and both stereoisomers is based on full scan mass spectra (Table 4). Furthermore, JA was identified at R_t 9.127 min by full scan mass spectrum [2].

Secale cereale. Young and premature caryopses of rye were extracted and purified in the usual manner and after methylation subjected to GC-MS or GC-SIM. At R_t 12.72 min the characteristic ions of JA-Me were obtained [2]. At R_t 12.80 min a full scan mass spectrum (*m/z* (rel. int.): 226 $[M]^+$ (2), 195 (11), 156 (30), 153 (27), 127 (9), 110 (9), 96 (12), and 83 (100)) corresponded to 9,10-dihydro-JA-Me [15], at R_t 13.08 min the GC-SIM of *m/z* 134.11 and 152.12 corresponded with authentic 6-*epi*-CA-Me, and at R_t 13.42 min the GC-SIM of *m/z* 134.11, 153.13, and 208.15 corresponded to authentic CA-Me (cf. Table 4). Thus, in actively growing caryopses of rye JA, 9,10-dihydro-JA, 6-*epi*-CA and CA could be identified.

Likewise at the premature stage II JA (R_t 12.62 min), dihydro-JA (12.72) and CA (13.42) were identified by GC-SIM. While JA dominated at stage I, at stage II CA dominated over the other JA related components.

DISCUSSION

The JA derivatives CA, 6-*epi*-CA, 7-*iso*-CA, and 6-*epi*-7-*iso*-CA were obtained from JA and 7-*iso*-JA by reduction of the keto group at C-6. The isomers can be separated by HPLC. Physical data (optical rotation, ^{1}H NMR and MS) allow clear discrimination between all four compounds.

In the past, only in the case of CA were all the physical data for structural characterization known [10], while for 6-*epi*-7-*iso*-CA-Me only the mass spectral fragmentation ions were reported [11, 12]. The optical rotation in CA described by [10] was slightly higher in comparison with our value (Table 3), while the assignable signals in the ^{1}H NMR spectrum of CA-Me for carbomethoxyl protons (δ3.65, 3H, *s*), hydroxy-methine proton (δ3.98, 1H, *m*) and olefinic protons (δ5.40, 2H, *m*) corresponded almost completely (Table 5).

The natural occurrence of 6-OH-analogues of JA is summarized in Table 6. With the exception of 7-*iso*-CA, all the isomers have been identified in plants. 6-*epi*-CA only occurs in small amounts and never appears as a major component. Obviously, the *cis* arrangement of substituents at C-6 and C-7 is an unfavoured configuration. In contrast, 6,7-*trans* arranged isomers (CA, 6-*epi*-7-*iso*-CA) form the major natural components. Thus, CA dominates over the other isomers in *A. phyllitidis* and *S. cereale*, while 6-*epi*-7-*iso*-CA is the predominant isomer in *Equisetum arvense* [12] and *J. regia*. Possibly, each species favours endogenously the pathway to JA or 7-*iso*-JA and as a consequence the metabolism, especially at

Table 6. Natural occurrence of CA and its 6,7-stereoisomers in plants and fungi

JA derivative	Natural occurrence	Ref.
CA	*Cucurbita pepo* seeds	[9, 10]
	Botryodiplodia theobromae culture filtrate	[14]
	A. phyllitidis spores	
	S. cereale immature caryopses	
6-*epi*-CA	*A. phyllitidis* spores	
	J. regia female flowers	
	S. cereale immature caryopses	
7-*iso*-CA	not detected as native compound	
6-*epi*-7-*iso*-CA	*Vicia faba* young fruits	[11]
	Equisetum arvense fertile fronds	[12]
	A. phyllitidis spores	
	J. regia female flowers	

senescence or maturation, leads to the corresponding isomer with reduced keto group at C-6, CA or 6-*epi*-7-*iso*-CA respectively. CA and its 6,7-isomers have been shown by TLC (system 3) not to be affected by conditions used for the extraction of plant material. Thus the different amounts of *cis* and *trans* isomers are not a consequence of the extraction procedure.

The pathway to these JA derivatives obviously takes place at different developmental stages. While in young tissues small amounts of these derivatives occurred (*Vicia faba* [11], *S. cereale*), tissues in the process of senescence or ripening contain large amounts of these derivatives in comparison to JA (*E. arvense* [12], *S. cereale*). The question arises whether JA is the real and only native regulator of senescence in plants or are other related compounds involved [16]? Despite the detection of JA-Me as a senescence-inducing compound [1] and the fundamental investigations on senescence induction by JA and JA-Me [17, 18, and refs therein], the high JA levels found especially in young tissues [19, 20], and the abundant occurrence of JA derivatives in older tissues, allow the assumption that the physiological role of JA in plant regulation is not restricted to the promotion of senescence. Possibly, CA or 6-*epi*-7-*iso*-CA are involved in the native regulation of senescence.

In *S. cereale*, we detected 9,10-dihydro-JA. Whether the configuration at C-7 is the native one is uncertain, because it is known that increasing injection temperature in GC induces the isomerization of 7-*iso*-JA-Me to JA-Me [21]. The same may also be true for 9,10-dihydro-JA-Me. The 9,10-dihydro-JA was found to occur endogenously in *Vicia faba* [11] and its isomer 9,10-dihydro-7-iso-JA was formed by the fungus *Botryodiplodia theobromae* [14].

EXPERIMENTAL

Preparation of JA analogues

(a) *Fermentation.* The fungus *Botryodiplodia theobromae* Pat. (syn. *Lasiodiplodia theobromae* Griff. & Maubl.) was used for the production of JA and 7-*iso*-JA [22, 14]. The strain i.1695-M isolated from Cuban grapefruit was kindly supplied by Dr O. Miersch. The fermentation conditions were as described in ref. [14]. The fungus was grown in surface culture at 30° in darkness.

The formation of 7-*iso*-JA and JA started on the days 3 and 5 of fermentation respectively [23]. For the preparation of 7-*iso*-JA (containing only minute amounts of JA), the fermentation (monitored by TLC) was stopped after the day 3 (or 4); for the preparation of JA the culture was grown for 2 weeks.

(b) *Isolation for JA.* The culture medium (14 days fermentation) from 10 flasks (each 150 ml) was frozen, lyophilized and extracted with EtOAc. The solvent extract was partitioned with satd Na_2CO_3 soln, the aq. phase acidified (pH 3) and repartitioned with EtOAc. The first purification of the crude and dried EtOAc extract (450 mg) was performed on silica gel (60 g, 2 × 40 cm) eluted with a stepwise gradient of EtOAc in $CHCl_3$. Frs of solvent mixt. 3:7–7:3 containing JA/7-*iso*-JA were combined (*ca* 100 mg crude extract) and rechromatographed on a column (1.1 × 45 cm) of DEAE-Sephadex A-25 according to ref. [24]. JA/7-*iso*-JA was eluted with 0.25 M HOAc in 80% MeOH (2.7 mg), isomerized by 1 M NaOH (see below) and purified by prep. HPLC [MeOH–HOAc (0.2%), 11:9], R_{tJA} 8.3 min) yielding 10.5 mg JA, which was stored under N_2 at 4°.

In order to obtain 7-*iso*-JA the fermentation was stopped after 4 days (TLC, system 1) yielding *ca* 660 mg crude extract containing 7-*iso*-JA and JA in the ratio of *ca* 9:1. After silica gel chromatography, 120 mg of 7-*iso*-JA/JA-mixture were obtained.

In order to get JA for the preparation of its derivatives the EtOAc extract was purified on a silica gel column. The JA/7-*iso*-JA fr. was isomerized by 1 M NaOH treatment at room temp. for 30 min [14]. The acidified (pH 3) reaction mixt. was partitioned against $CHCl_3$ resulting in a JA/7-*iso*-JA-mixture in the equilibrium ratio of 9:1 [25].

(c) *Preparation of JA derivatives.* The prepurified (silica gel column) JA fraction was dissolved in MeOH and treated with $NaBH_4$ until complete reaction (monitored by TLC, system 2). After adding HOAc, the reaction mixt. was diluted with H_2O and partitioned (pH 3) against EtOAc followed by evapn of the organic phase. The 7-*iso*-JA fraction, containing JA, was similarly reduced. The reaction products were methylated with CH_2N_2 and purified by silica gel chromatography (40 g, 1.6 × 50 cm, *n*-hexane–EtOAc–HOAc (60:40:1). 6-*epi*-CA-Me and 7-*iso*-CA-Me could only be partially separated eluting between 85 and 104 ml, and 100 and 112 ml respectively, while 6-*epi*-7-*iso*-CA-Me and CA-Me eluted together between 116 and 134 ml. The final purification of the derivatives was performed by HPLC. The hydrolysis of the methyl esters was achieved using 1 M NaOH in MeOH–H_2O (1:1) overnight at room temp. and subsequent partitioning (pH 3) with $CHCl_3$.

TLC: silica gel GF_{254} was used (layer: 0.3 mm) with the following systems: (1) $CHCl_3$–MeOH–H_2O (140:20:1) [4], (2) $CHCl_3$–EtOAc–Me_2CO–HOAc (40:10:5:1), (3) *n*-hexane–EtOAc–HOAc (60:40:1) [4]. Detection was performed with anisaldehyde reagent (HOAc–H_2SO_4–anisaldehyde, 100:2:1 [26], 110°, 10 min). HPLC: HPLC purification was performed on Lichrosorb RP 18 7 μm (10 × 250 mm), flow rate 4 ml min^{-1}, detector set at 228 nm (JA) or 214 nm (JA derivatives) with MeOH–HOAc (0.2%) (11:9) for JA or 1:1 for free acids or with MeOH–H_2O (11:9) for methyl esters. GC-MS: system 1: GC was performed on a 25 m × 0.2 mm cross-linked methylsilicone fused silica column, film thickness 0.11 μm (Hewlett-Packard Ultra 1), splitless injection, He as carrier gas (2.5 ml min^{-1}) and the following temp. programme: from 50° (1 min) to 140° (25° min^{-1}) and from 140° (1 min) to 160° (2.5° min^{-1}). Temp.: injection −275°, direct inlet interface −230° and ion source −250°. The electron impact energy was 70 eV. System 2: GC was performed under the same conditions as system 1 applying the following temperature programme: from 60° (1 min) to 110° (25° min^{-1}) and from 110° (1 min) to 270° (10° min^{-1}). The electron impact energy was 70 eV.

^1H NMR: the methyl esters of the JA derivatives were measured in pyridine-d_5 at 250 MHz and TMS (int. standard) was set at 0. Optical rotation: the $[\alpha]_D$ values were determined in MeOH.

Isolation of CA and its 6,7-stereoisomers from different plant sources

(a) *Plant materials.* Female flowers (644 flowers, 12.8 g fr. wt) of walnut (*Juglans regia* L.) were collected early in May 1978, about 20 days before anthesis of catkins, lyophilized and stored until extraction at −20°. Caryopses of spring rye (*Secale cereale* L. cv Petka) were harvested in 1989 at two developmental stages: I, during intensive fruit growth (1300 caryopses, 49.0 g fr. wt, 37.7 ± 5.8 mg/caryopsis), II, late milky ripeness (1200 caryopses, 74.3 g fr. wt, 61.7 + 8.6 mg/caryopsis), put into MeOH and stored at −20°. Spores of *Anemia phyllitidis* L. Sw. (6.2 g fr. wt) were kindly supplied in 1988 by Prof. Dr H. Schraudolf (University of Ulm, General Botany, Ulm, Germany).

(b) *Extraction, purification and identification.* Plant materials were homogenized in 80% MeOH (× 3) using a blendor (walnut,

rye) or in a mortar with Al_2O_3 (*Anemia* spores). After evaporating the MeOH, the aq. residue was frozen, thawed and centrifuged. The supernatants were partitioned at pH 3 with EtOAc. These extracts were dried, evapd and chromatographed on DEAE-Sephadex A-25 as described previously [27]. Frs containing JA and JA derivatives (0.25 M HOAc in 80% MeOH) were pooled and these frs of the walnut extract were purified by TLC (system 2). R_f zones 0.52–0.64 for JA (R_f 0.60) and 0.37–0.48 for ABA (R_f 0.41) were eluted with MeOH and adhering silica gel was removed by cotton wool filtration. The rye extracts were chromatographed on a silica gel column (0.8 × 22 cm, eluted with *n*-hexane–EtOAc–HOAc (60:40:1); frs 1–10 of 2 ml, frs 11 and 12 of 10 ml). Identification of JA derivatives was performed by GC-MS using system 1 for *J. regia* and *A. phyllitidis* or system 2 for *S. cereale* (Table 4).

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