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SYNTHESIS AND BIOLOGICAL EFFECTS OF AROMATIC ANALOGS OF ABSCISIC ACID

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Key Word Index—Abscisic acid; 3-methyl-5-aryl-2,4-pentadienoic acids; biological activity; germination of lettuce seed; acceleration of *Tropaeolum* leaf discs senescence; inhibition of wheat coleoptile elongation; inhibition of transpiration.

Abstract—Thirty six different 3-methyl-5-aryl-2,4-pentadienoic acids and esters were synthesized using the Reformatsky and Wittig reactions. The different geometrical isomers were conveniently separated by the dry column technique. Assignment of configuration of the pentadienoic side chain was based on NMR and UV properties. The biological activities of the aromatic analogs of ABA were determined in four bioassays. Most of the analogs were less active than the natural hormone. Only 3-methyl-5-*p*-chlorophenyl Δ^2-*trans*,Δ^4-*trans*-pentadienoic acid exhibited high ABA-like activity in all four bioassays.

INTRODUCTION

Recent works have furnished evidence that the plant hormone abscisic acid (ABA) plays a major role in regulating the water balance in plants and in their adaptation to water shortages [1–3]. Different stress conditions such as water deficits [4], salinity and high osmotic pressure [1], decline in plant turgor [5], mineral deprivation [6] and water stress induced by the fungus *Verticillium* [7] cause an increase in the ABA content of leaves. It appears that the hormone improves the plant water balance by the closure of stomata [8, 9] as well as by the increase of root water permeability [10]. The question has been raised whether it would be possible, by ABA application, to increase plant growth under stress conditions.

In preliminary experiments [11] it was shown that ABA sprayed on barley and wheat seedlings subjected to water deprivation, delayed the appearance of drought-induced senescence and greatly extended the period of plant survival. In addition, treatment with ABA increased the amount of dry matter produced, indicating an effect on the efficiency of water utilization [12]. When conditions for normal growth were restored, the plants treated with ABA resumed growth whereas the control plants did not recover [11].

Thus, ABA can be suggested as an anti-stress agent especially in environments with a short rainy season and a correspondingly long dry season. Many other uses of ABA in modern agriculture have already been suggested [3], however its high cost, resulting from a long and difficult synthesis with low overall yield [13, 14], makes wide use impractical. Moreover, ABA has a high turnover rate and undergoes rapid deactivation under field conditions [11] by transformation into the inactive isomer Δ^2-*trans*, Δ^4-*trans* ABA. Those facts stimulated intensive investigations with the aim of synthesizing simpler but active analogs of ABA.

The objectives of this investigation were to synthesize and study the biological activity of some additional ABA analogs. Investigations which correlate the biological activity of a number of ABA analogs with their chemical structure, indicated that the special configuration of the side chain Δ^2-*cis*, Δ^4-*trans*-pentadienoic acid might be a prerequisite for high activity [3]. The essential features of the cyclohexane ring are less defined and some changes can be made without much altering the hormonal activity. The present paper describes the synthesis and biological testing of ABA analogs in which the pentadienoic acid is attached to a variety of aromatic residues.

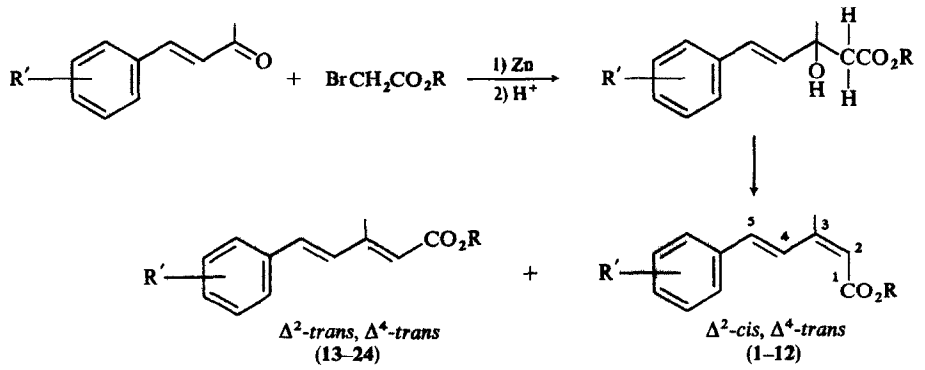
RESULTS AND DISCUSSION

Synthesis of ABA analogs via the Reformatsky reaction

Different benzalacetones were prepared by condensing aromatic aldehydes with acetone under basic conditions. Further condensation of the substituted benzalacetones with alkylbromoacetate in the presence of zinc [15] yielded the appropriate β-hydroxyesters (scheme 1). Acid catalyzed dehydration of the β-hydroxyesters gave the appropriate 2,4-pentadienoic esters. Since the double bond in the benzalacetones is in the *trans* configuration and the dehydration step is not stereospecific, a mixture of the two isomers—Δ^2-*cis*, Δ^4-*trans* and Δ^2-*trans*, Δ^4-*trans* pentadienoic esters is obtained. The ratio between the two isomers differs from reaction to reaction, but the percent of the Δ^2-*trans*, Δ^4-*trans* isomer is always higher. Upon basic hydrolysis, the esters were transformed into the corresponding acids. Analytical data of 24 analogs thus obtained is summarized in Tables 1 and 2.

Synthesis of ABA analogs via the Wittig reaction

The Wittig reaction [16] results in the formation of a carbon–carbon double bond by reaction of alkylidenetriphenylphosphoranes with aldehydes or ketones. The method used by us for the synthesis of aromatic analogs of ABA included the following steps: (a) treatment of β,β-dimethyl ethylacrylate with *N*-bromosuccinimide which



Scheme 1. Preparation of pentadienoic acid derivatives via the Reformatsky reaction.

led to the formation of ethyl-4-bromo-3-methyl-2-butenoate; (b) the bromoester was then treated with triphenylphosphine to yield the quaternary triphenylphosphonium bromide. Since the bromoester was composed of a mixture of the two geometrical isomers, the phosphonium salt derived is a mixture of the *cis* and *trans* isomers; (c) treatment with sodium ethoxide in ethanol removed a proton α to the positive phosphorus to give a phosphorane which reacted rapidly with the appropriate aromatic aldehyde yielding 2,4-pentadienoic ester derivatives and triphenylphosphine oxide. A mixture of *cis* and *trans* isomers usually results from the Wittig reaction [17], and in our case a mixture of four geometrical isomers is theoretically expected (scheme 2). The condensation products can in turn be hydrolyzed to give the appropriate carboxylic acids. In following the above route we

Table 1. 5-Aryl 3-methyl Δ^2-*cis*, Δ^4-*trans*-pentadienoic ethyl esters and acids prepared by the Reformatsky reaction

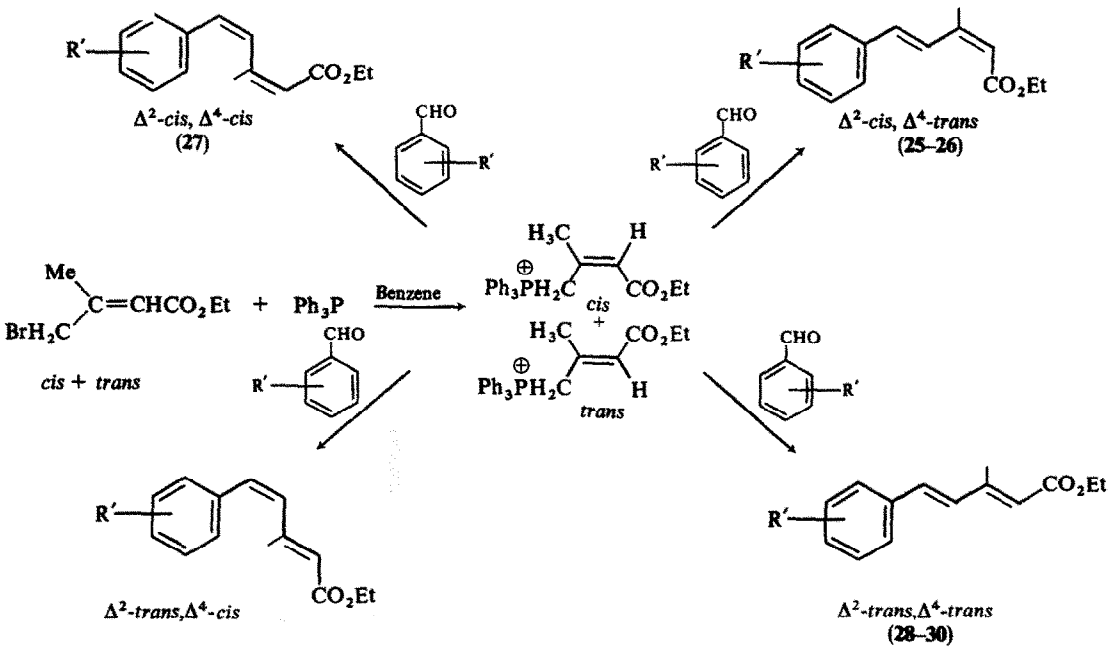
Compound	R	R'	Yield (%)	bp/mp (°)	C% Calcd.	C% Found	H% Calcd.	H% Found	Cl% Calcd.	Cl% Found	UV absorptions λ_{max}^{MeOH}(nm)($10^{-4}\varepsilon$)
(1)	Et	*o*-Me	36	182/1.5 mm	78.23	78.01	7.88	7.98	—	—	227(1.15); 309(2.3)
(2)	Et	*m*-Me	28	135/3 mm	78.23	78.20	7.88	7.97	—	—	232(1.23); 308(2.65)
(3)	Et	*p*-Me	32	28	78.23	78.39	7.88	7.80	—	—	230(1.6); 317(3.27)
(4)	Et	*m*-MeO	38	172/2 mm	73.14	72.57	7.37	7.25	—	—	210(1.74); 304(1.95)
(5)	Et	*p*-Cl	36	195/0.2 mm	67.06	67.15	6.03	6.08	12.76	12.91	228(1.4); 312(2.7)
(6)	Et	2,4-di-Cl	37	51	58.96	58.77	4.95	4.96	24.91	25.03	234(1.25); 311(2.31)
(7)	H	*o*-Me	*	180–81†	77.20	76.89	6.98	7.19	—	—	
(8)	H	*m*-Me	*	166–67†	77.20	77.09	6.98	6.96	—	—	
(9)	H	*p*-Me	*	179–81†	77.20	77.06	6.98	6.89	—	—	
(10)	H	*m*-MeO	*	129–31†	71.54	71.75	6.47	6.39	—	—	
(11)	H	*p*-Cl‡	*	210–12¶	—	—	—	—	—	—	
(12)	H	2,4-di-Cl	*	200–0.1	56.05	55.95	3.92	4.06	27.63	27.54	

* Crude yields are almost quantitative and after recrystallization ~90%; † Recrystallized from ethylether–hexane; ‡ Identical with compound from ref. [15]; § Recrystallized from benzene; ¶ Recrystallized from methanol.

Table 2. 5-Aryl 3-methyl Δ^2-*trans*,Δ^4-*trans*-pentadienoic ethyl esters and acids prepared by the Reformatsky reaction

Compound	R	R'	Yield (%)	bp/mp (°C)	C% Calcd.	C% Found	H% Calcd.	H% Found	Cl% Calcd.	Cl% Found	UV absorptions λ_{max}^{MeOH}(nm)($10^{-4}\varepsilon$)
(13)	Et	*o*-Me	48	182/1.5 mm	78.23	78.26	7.88	7.95	—	—	228(1.08); 308(2.8)
(14)	Et	*m*-Me	39	135/3 mm	78.23	78.30	7.88	7.75	—	—	232(1.24); 308(3.14)
(15)	Et	*p*-Me	45	65–66*	78.23	78.33	7.88	7.81	—	—	233(1.06); 315(3.6)
(16)	Et	*m*-MeO	52	173/2 mm	73.14	73.03	7.37	7.37	—	—	209(1.81); 304(2.4)
(17)	Et	*p*-Cl	45	79–80*	67.06	66.80	6.03	6.15	12.76	13.92	228(1.32); 311(3.13)
(18)	Et	2,4-di-Cl	48	88–89†	58.96	59.06	4.95	4.98	24.91	24.87	235(1.38); 310(2.84)
(19)	H	o-Me	‡	148–50	77.20	77.36	6.98	7.03	—	—	
(20)	H	*m*-Me	‡	153	77.20	77.18	6.98	6.88	—	—	
(21)	H	*p*-Me	‡	188–90	77.20	77.34	6.98	7.09	—	—	
(22)	H	*m*-MeO	‡	175	71.54	70.48	6.47	6.53	—	—	
(23)	H	*p*-Cl	‡	190–94§	—	—	—	—	—	—	
(24)	H	2,4-di-Cl	‡	185	56.05	55.80	3.92	3.75	27.63	27.83	

* Recrystallized from hexane; † Recrystallized from hexane–methylene chloride; ‡ Crude yields are almost quantative and after recrystallization ~90%; § Identical with compound previously prepared [12].



Scheme 2. Preparation of pentadienoic acid esters via the Wittig reaction.

were able to synthesize different analogs of ABA as summarized in Table 3. The various isomers were readily isolated and purified by preparative 'dry column' chromatography [19] (see Experimental section) using deactivated alumina as absorbent. Characterization and assignment of configuration were achieved by spectroscopic methods. Total yields reached 80%, which consisted of 20–35% of the Δ^2-*cis*, Δ^4-*trans* isomers, 40–55% of the Δ^2-*trans*, Δ^4-*trans* isomers and only minute quantities of the other two isomers. In one case only, with 2,4,6-trimethoxybenzaldehyde, an appreciable amount of the Δ^2-*cis*, Δ^4-*cis* isomer was obtained. This might have been caused by high reactivity of the aldehyde which enhanced elimination of triphenylphosphine oxide or to steric factors which favour *cis*-olefination. It is known [20] that the planar conformation is preferred with the Δ^4-*trans* isomers, whereas in the case of Δ^4-*cis* isomers, the aromatic ring and the side chain are in different planes. In the case of the 2,4,6-trimethoxy derivative the two methoxy groups in the ortho position cause severe steric interference, in the planar conformation of the Δ^4-*trans* isomer, and as a result the thermodynamic stability of the usually less stable Δ^4-*cis* isomer approaches that of the *trans* isomer.

Characterization and structure assignment

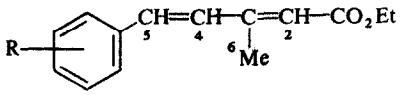
Structure elucidation was based on the NMR properties of similar isomeric systems [18, 20, 21]. The NMR data obtained for the different isomeric esters are summarized in Table 4. From all these data it can be concluded as follows: (a) The C-2 proton appears as a quartet in all isomers but at somewhat higher field ($\delta \sim 5.7$) in the Δ^2-*cis* isomers compared to the Δ^2-*trans* isomers ($\delta \sim 5.9$). (b) The olefinic protons, C-4 and C-5 appear as unsymmetrical doublets in the Δ^4-*cis* isomers with a coupling constant $J = 12.5$ Hz, characteristic of *cis*-disubstituted olefins. The corresponding value in the Δ^2-*cis*, Δ^4-*trans* compounds is higher ($J \sim 16$ Hz), while in the Δ^2-*trans*,

Table 3. 5-Aryl 3-methylpentadienoic ethyl esters and acids prepared by the Wittig reaction

Compound	R	R′	Configuration	Yield (%)	bp/mp (°)	C% Calcd.	C% Found	H% Calcd.	H% Found	UV absorptions λ_{max}^{MeOH}(nm)($10^{-4}\varepsilon$)
(25)	Et	*o*-MeO	Δ^2-*cis*,Δ^4-*trans*	35	190/2 mm	73.14	72.96	7.37	7.22	230(0.97); 333(1.5)
(26)	Et	*p*-MeO	Δ^2-*cis*,Δ^4-*trans*	34	62–64	73.14	72.90	7.37	7.45	235(1.24); 331(2.7)
(27)	Et	2,4,6-tri-MeO	Δ^2-*cis*,Δ^4-*cis*	21	96–98	66.65	66.80	7.24	7.14	247(1.64); 334(1.2)
(28)	Et	*o*-MeO	Δ^2-*trans*,Δ^4-*trans*	43	190/2 mm	73.14	72.69	7.37	7.10	229(0.98); 330(1.94)
(29)	Et	*p*-MeO	Δ^2-*trans*,Δ^4-*trans*	46	40–42	73.14	73.12	7.37	7.49	235(1.18); 327(2.97)
(30)	Et	2,4,6-tri-MeO	Δ^2-*trans*,Δ^4-*trans*	54	94–95	66.65	66.55	7.24	7.19	246(1.34); 343(2.3)
(31)	H	*o*-MeO	Δ^2-*cis*,Δ^4-*trans*	*	164–66	71.54	71.41	6.47	6.48	
(32)	H	*p*-MeO	Δ^2-*cis*,Δ^4-*trans*	*	149†					
(33)	H	2,4,6-tri-MeO	Δ^2-*cis*,Δ^4-*cis*	*	152	64.73	64.98	6.52	6.40	
(34)	H	*o*-MeO	Δ^2-*trans*,Δ^4-*trans*	*	190–91	71.54	71.65	6.47	6.40	
(35)	H	*p*-MeO	Δ^2-*trans*,Δ^4-*trans*	*	178–80†					
(36)	H	2,4,6-tri-MeO	Δ^2-*trans*,Δ^4-*trans*	*	183–85	64.73	65.11	6.52	6.64	

* Yields are almost quantiative, and ~90% after recrystallization; † Identical with compound previously prepared [15].

Table 4. NMR spectra of 3-methyl-5-arylpenta-2,4-dienoic acid ethyl esters*



Compound	C-2	C-4	C-5	C-6†	Configuration
(1)	5.72*q*‡	8.31*d*§	7.11*d*§	2.10*d*	Δ^2-*cis*,Δ^4-*trans*
(2)	5.67*q*	8.36*d*§	6.80*d*	2.10*d*	Δ^2-*cis*,Δ^4-*trans*
(3)	5.72*q*	8.36*d*§	6.84§	2.10*d*	Δ^2-*cis*,Δ^4-*trans*
(4)	5.76*q*	8.43*d*§	6.88§	2.13*d*	Δ^2-*cis*,Δ^4-*trans*
(5)	5.71*q*	8.35*d*§	6.70*d*§	2.06*d*	Δ^2-*cis*,Δ^4-*trans*
(6)	5.78*d*	8.42*d*§	7.15*d*§	2.13*d*	Δ^2-*cis*,Δ^4-*trans*
(13)	5.86*q*	obs.	obs.	2.40*d*	Δ^2-*trans*,Δ^4-*trans*
(14)	5.85*q*	6.80*s*	6.80*s*	2.36*d*	Δ^2-*trans*,Δ^4-*trans*
(15)	5.83*q*	6.75*d*¶	6.75*d*¶	2.36*d*	Δ^2-*trans*,Δ^4-*trans*
(16)	5.93*q*	6.85*s*	6.85*s*	2.42*d*	Δ^2-*trans*,Δ^4-*trans*
(17)	5.86*q*	6.77*d***	6.77*d***	2.37*d*	Δ^2-*trans*,Δ^4-*trans*
(18)	5.91*q*	6.87*s*	6.87*s*	2.45*d*	Δ^2-*trans*,Δ^4-*trans*
(27)	5.66*q*¶	7.30*d*††	6.42*d*††	1.77*d*	Δ^2-*cis*,Δ^4-*cis*
(28)	5.86*d*‖	obs.	obs.	2.43*d*	Δ^2-*trans*,Δ^4-*trans*
(29)	5.81*q*	6.76*s*	6.76*s*	1.88*d*	Δ^2-*trans*,Δ^4-*trans*
(30)	5.65*q*	7.27*s*	7.27*s*	2.42*d*	Δ^2-*trans*,Δ^4-*trans*

* Runs at 60 MHz in $CDCl_3$ solutions using TMS as internal standard. Values are δ in ppm; † $J = 1 - 2$ Hz; ‡ *s* singlet, *d* doublet, *q* quartet; § The line separations or coupling constant (*J*) are 15–16 Hz; ‖ $J = 1$ Hz; obs, superimposed or obscured signal; ¶ $J = 2$ Hz; ** $J = 2.5$ Hz; †† $J = 13$ Hz.

Δ^4-*trans* isomers, protons at C-4 and C-5 are almost equivalent (broad quartet or a doublet with $J \sim 1$ Hz). (c) The signal of the olefinic C-4 proton is shifted downfield (δ, 8.3–8.4) in the Δ^2-*cis*, Δ^4-*trans* isomers, lower than in all the other isomers, which may be attributed to planar conformation. (d) The methyl group (C-6) displays a doublet at high field (δ, 2.1) in the Δ^2-*cis* isomers, and at somewhat lower field ($\delta \sim 2.4$) in the Δ^2-*trans* isomers. The Δ^4-*cis* compounds are characterized by a signal of the 3-methyl group at higher field than in the Δ^4-*trans* isomers.

NMR spectral data led to the assignment of the configuration Δ^2-*cis*, Δ^4-*trans*, to compounds **1–6**, Δ^2-*trans*, Δ^4-*trans*, to compounds **13–18**, **28–30**, and Δ^2-*cis*, Δ^4-*cis* to compound (**27**).

The conclusions drawn from the NMR studies are consistent with UV data. Absorptions due to two bonds appear in the spectra of the isomeric esters; the first between 305–315 nm (high molar extinction) and the second between 210–235 nm (lower molar extinction). The intensity of the first absorption is lower for the Δ^2-*cis*, Δ^4-*trans* isomers than that for the appropriate Δ^2-*trans*, Δ^4-*trans* isomers. The case is usually reversed for the intensity of the second absorption [22, 23].

The isomer Δ^2-*cis*, Δ^4-*cis* (**27**) gave an entirely different spectrum than the other isomers. Here the ε_{max} at 334 nm is lower than the ε_{max} at 247 nm. In addition, a hypsochromic shift was observed with this isomer (λ_{max} 334) as compared with the appropriate Δ^2-*trans*, Δ^4-*trans* isomer (λ_{max} 343). This shift might be ascribed to steric inhibition of resonance and is an additional proof for the nonplaner structure of the Δ^2-*cis*, Δ^4-*cis* isomers [20].

Table 5. Bioassay I. Effects of aromatic ABA analogs on the germination of lettuce seed

Compound*	% Seed germination†			
	10^{-4}M	10^{-5}M	10^{-6}M	10^{-7}M
ABA	0‡	20	66	80
(7)	70	79	75	79
(9)	78	80	83	89
(11)	74	96	96	98
(19)	74	82	90	93
(21)	68	72	78	85
(23)	62	74	90	96
(24)	57	62	75	82

* Only compounds that showed some kind of activity were recorded; all other acids (Tables 1, 2, 3) were inactive.

† Percent germinated in water control was 98.

‡ The numbers represent an average of two replicates (100 lettuce seeds).

Biological activity

The biological activities of the aromatic analogs of ABA were determined by the following bioassays: germination of lettuce seeds [24] (bioassay I); acceleration of *Tropaeolum* leaf disc senescence [25] (bioassay II); inhibition of wheat coleoptile elongation [26, 27] (bioassay III), and inhibition of transpiration (bioassay IV). Inhibition of germination by ABA is one of the easiest responses to measure. With the aromatic analogs, growth inhibition does not necessarily show that the compounds act in the same reactions effected by the natural hormone. Table 5 shows the effect of the aromatic ABA analogs on the germination of lettuce seed, as compared with water and ABA treatments.

It can be seen that the most active compound was **24** causing inhibition, just one order of magnitude lower than ABA itself. Somewhat lower activity was exhibited by compounds **19**, **21**, **23** and compound **11** showed weak activity only at high concentration. With compound **7** irregularities were observed causing the same inhibition

Table 6. Bioassay II. Effect of aromatic ABA analogs on the extent of acceleration of *Tropeolum* leaf discs senescence as measured by chlorophyll content

Compound*	Average acceleration of senescence (%)			
	10^{-4}M	10^{-5}M	10^{-6}M	10^{-7}M
ABA	70†	63	45	33
(8)	−17‡	−26	−16	−21
(21)	30	13	2	5
ABA	42	34	20	12
(10)	45	44	2	4
ABA	76	71	59	50
(20)	50	43	26	15
ABA	47	27	10	8
(23)	53	12	12	6
ABA	37	23	22	6
(31)	−13	−48	−52	−16
ABA	63	57	55	40
(35)	34	−5	−10	−7
ABA	38	32	20	8
(33)	9	18	14	16
(36)	−14	−28	−26	12

* Only compounds that showed some kind of activity were recorded. † The figures indicate the extent of chlorophyll degradation as compared to water controls (average of six replicates). ‡ Values with negative sign indicate retardation of chlorophyll breakdown.

at all concentrations. All other compounds were inactive when tested at concentrations up to 10^{-4}M.

Chlorophyll content is related to the degree of senescence of leaf tissue and ABA promotes the loss of chlorophyll in leaf discs [3]. The effect of the aromatic ABA analogs on the senescence response in leaf discs of *Tropaeolum* is summarized in Table 6. For each group of test compounds, water and ABA controls were run. To enable comparison of activities (as different values were obtained from controls run on different days), we defined

Table 7. Bioassay III. Effect of aromatic ABA analogs on the extent of inhibition of wheat coleoptile elongation

Compound*	Average growth inhibition (%)			
	10^{-4}M	10^{-5}M	10^{-6}M	10^{-7}M
ABA	94†	83	71	27
(8)	20	10	11	10
(12)	29	11	8	2
(24)	23	2	4	3
ABA	78	66	6	0
(9)	−19‡	−13	−3	−2
ABA	98	83	63	53
(11)	53	45	44	44
(23)	61	40	40	40
ABA	96	83	58	31
(22)	−26	−18	−6	−1
(32)	20	18	16	15
ABA	70	61	32	5
(34)	5	−23	−43	−32
ABA	74	68	51	29
(36)	19	13	11	10

* Only compounds that showed activity were recorded, all other pentadienoic acids were inactive. † Inhibition of coleoptile elongation compared with buffer controls (average of 4–6 replicates). ‡ Values with negative sign indicate acceleration of coleoptile elongation.

Table 8. Bioassay IV. Effect of aromatic ABA analogs on the extent of inhibition of transpiration in barley leaves

Compound*	Average inhibition of transpiration (%)			
	10^{-4}M	10^{-5}M	10^{-6}M	10^{-7}M
ABA	67†	54	30	12
(7)	24	21	21	15
(8)	27	18	21	9
(9)	30	27	24	15
ABA	67	50	38	33
(11)	33	33	24	10
(22)	21	24	19	18
ABA	68	46	33	15
(12)	28	20	12	2
(21)	31	17	15	4
ABA	59	50	32	20
(23)	48	41	28	26
ABA	49	44	39	33
(33)	−22‡	−20	25	−22
(36)	−7	−20	0	−22
ABA	64	47	45	26
(34)	22	33	25	16
ABA	57	47	37	20
(35)	28	27	27	25

* Compounds **(10)**, **(20)**, **(24)**, **(31)**, **(32)** were completely inactive. † Percent of transpiration as compared to transpiration in water controls (average of five replicates). ‡ Negative numbers indicate enhancement of transpiration.

the "percent of acceleration of senescence" as follows: If A = chlorophyll extinction value obtained from discs treated with water, and B = chlorophyll extinction value from discs treated with test compound, then $(A - B/A) \times 100$ = percent of acceleration of senescence.

Compounds **10**, **20** and **23** (at high concentration) appear to be almost as active as ABA in promoting the loss of chlorophyll. The opposite effect, namely, retardation of chlorophyll breakdown is exhibited by compounds **8**, **31** and **36** which act in a similar way to cytokinin hormones. Of particular interest are the results obtained with the two geometrical isomers **33** (all *cis*) and **36** (all *trans*). While compound **33** accelerated chlorophyll degradation, its isomeric compound showed retardation activity.

Another bioassay in which the inhibitory effect of ABA is marked is the elongation of wheat coleoptiles. The effect of the aromatic ABA analogs in this test are shown in Table 7. As in bioassay II the "percent of growth inhibition" was similarly defined. If A = length of the coleoptile obtained in buffer control and B = length of coleoptile obtained with a test compound, then $(A - B/A) \times 100$ = percent of growth inhibition.

A relatively high inhibitory activity was exhibited by the two *p*-chlorophenyl isomers **11** and **23**. As the effect is equal at all concentrations, the activity might be attributed to some kind of nonspecific poisoning. Compounds **8**, **12**, **24** and **36** showed weak inhibitory activity only at a concentration of 10^{-4}M. IAA-like activity, causing acceleration of coleoptile growth was observed in compounds **9**, **22** and **34**.

ABA when supplied to cut leaves, causes closure of stomata and reduction of transpiration [8]. The effect of the aromatic ABA analogs on transpiration rates of barley leaves is summarized in Table 8. "Percent of inhibition of transpiration" was defined as in the former

Table 9. Summary of the biological activities found for aromatic analogs of ABA*

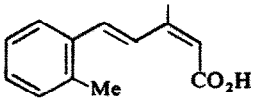
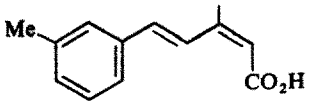
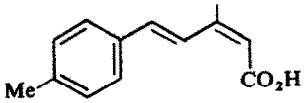
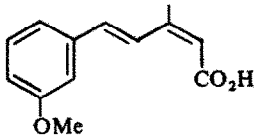
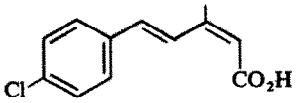
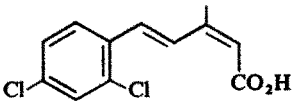
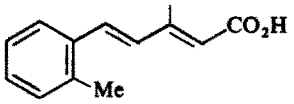
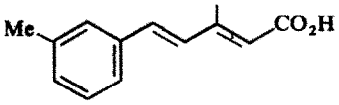
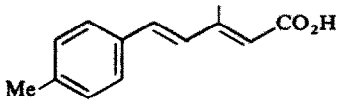
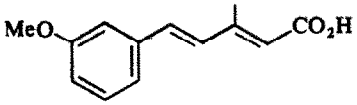
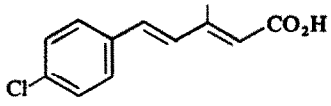
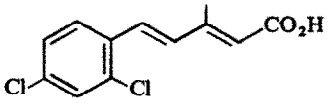
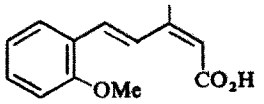
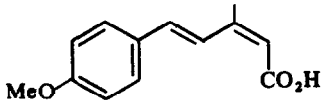
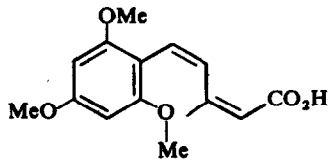
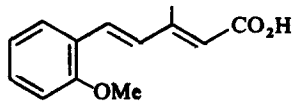
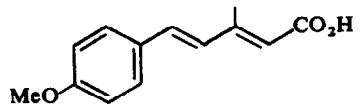
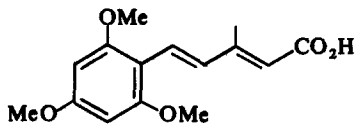
Compound	Structure	Bioassay I	Bioassay II	Bioassay III	Bioassay IV
(7)		+	0	0	+
(8)		0	–	+(a)	+
(9)		+	0	–	+
(10)		0	+(a)	0	0
(11)		+(a)	0	+	+
(12)		0	0	+(a)	+
(19)		+	0	0	0
(20)		0	+	0	0
(21)		+	+	0	+
(22)		0	0	–	+
(23)		+	+	+	+
(24)		+	0	+(a)	0
(31)		0	–	0	0

Table 9—(*cont.*)

Compound	Structure	Bioassay I	Bioassay II	Bioassay III	Bioassay IV
(32)		0	0	+	0
(33)		0	+	0	–
(34)		0	0	–	+
(35)		0	+(a)	0	+
(36)		0	–	+	–

* Key: + = ABA like activity; – = antagonistic effect to ABA; (a) = active only at high concentration; 0 = no activity.

bioassay and is expressed as a percent of the transpiration rate in water controls.

Almost all compounds showed activity in this bioassay which indicates high sensitivity but poor specificity of the test. Most compounds exhibited moderate inhibitory activity and only compound **23** (3-methyl, *p*-chlorophenyl Δ^2-*trans*, Δ^4-*trans* pentadienoic acid) appears to be almost as active as ABA. The two isomers **33** and **36** enhanced transpiration similarly to cytokinins.

A summary of biological activities found for the various aromatic ABA analogs is given in Table 9. A number of compounds are absolutely inactive but most of the analogs caused some visible effect in the test systems. In most cases the inhibitory activity was far inferior to that of ABA. Some analogs exhibited ABA-like activity in one bioassay but had no such activity in another assay. Moreover, some compounds which on one hand exhibited ABA-like activity such as suppression of coleoptile growth, exerted, on the other hand, effects which were in contrast with the usual influence of ABA on plant tissue, e.g. retarding chlorophyll degradation. Whereas isomerization of ABA to Δ^2-*trans* ABA causes almost complete inactivation, no such correlation could be observed in the aromatic analogs. On the contrary, some of the Δ^2-*trans* analogs exhibited higher inhibitory activity than their Δ^2-*cis* isomers. The nature of the substituent and its position on the benzene ring was not related in a predictable fashion with the biological activity. It has been already stated [3] that any change in the molecule of ABA decreases the growth inhibitory activity and the present work supports this rule. Only one compound, 3-methyl-5-*p*-chlorophenyl Δ^2-*trans*, Δ^4-*trans* pentadienoic acid (**23**) exhibited high ABA-like activity in all four bioassays. In two assays (I and III) its activity was two orders of magnitude less than ABA, but in the other two assays (II and IV) it was almost as active as ABA.

In spite of the lower activity of some of the aromatic ABA analogs, they are readily available and should prove useful in agricultural studies especially from the economic point of view.

EXPERIMENTAL

All mps were determined in an open capillary and are uncorr IR spectra were recorded in $CHCl_3$ (liquids) or in KBr discs (solids). UV spectra were recorded in absolute MeOH. The NMR spectra were taken in $CDCl_3$, at 100 MHz and chemical shifts are in ppm (δ) from TMS as internal standard. Benzalacetones were prepared by the method of Milborrow [28]. *p*-Chlorobenzalacetone was prepared according to Sondheimer *et al.* [29].

Separation of isomers on 'dry-column' chromatography. A column (20 mm i.d.) was packed dry with deactivated Al_2O_3. The Al_2O_3 (80–200 mesh) was deactivated by adding 6% H_2O and equilibrating under vacuum in a rotary evaporator for 5 hr [19]. If a mixture of solvents were used for the developing process, equilibration of the deactivated Al_2O_3 with the solvents was performed in the same way. The oily reaction mixtures were deposited on the adsorbent by dissolving in CH_2Cl_2, adding 5 × its wt. of deactivated Al_2O_3 and evaporating the mixture to dryness in a rotary evaporator at 40°. The compound-adsorbent mixture was then distributed evenly on the top of the column and covered with a small layer of sand. The development

process was complete when the solvent reached the bottom of the column (25–30 min.). Fractionation was continued and the isomeric compounds were separated by elution and collecting in the standard manner. The ratio of mixture to adsorbent was 1:250.

Ethyl 3-methyl-5-σ-tolylpenta-2,4-dienoate (**1**) *and* (**13**). A soln of ethylbromoacetate (4.6 g, 0.025 mol) in 10 ml dry C_6H_6 was gently refluxed with a hot mixture (80°) of σ-methylbenzalacetone (4.0 g, 0.025 mol) in 50 ml. dry C_6H_6 and acid-etched Zn granules (1.8 g, 0.0275 mol). The reaction mixture was refluxed for an additional 20 hr., cooled and shaken with 20 ml 20% HOAc. The mixture was extracted with Et_2O, and the Et_2O soln was successively washed with satd $NaHCO_3$ soln, satd NaCl soln and H_2O. The organic phase was dried over dry Na_2SO_4 and the solvent was evap. The oily residue was mixed with 50 ml C_6H_6, *p*-toluenesulfonic acid (0.2 g) added and the soln refluxed in a Dean–Stark H_2O trap. When all the H_2O was collected, the mixture was extracted with satd NaCl soln and dried over dry Na_2SO_4. The solvent was evap to give an oily residue which was composed (TLC-spots) of two products and starting materials. NMR analysis of the oil (based on peak areas) showed a proportion of approximately 36% of the Δ^2-*cis*,Δ^4-*trans* isomer (**1**) and 48% of the Δ^2-*trans*,Δ^4-*trans* isomer (**13**). The isomeric esters were isolated by the 'dry column' technique with C_6H_6–hexane (3:7). Compounds **2–6** and **14–18** were prepared by the same procedure. Elementary analysis, physical and spectroscopic properties are summarized in Tables 1 and 2, and NMR data in Table 4.

3-Methyl-5-σ-tolylpenta-Δ^2*-cis,*Δ^4*-trans dienoic acid* (**7**). Ethyl 3-methyl-5-σ-tolypenta-Δ^2-*cis*,Δ^4-*trans* dienoate (**1**) (130 mg.) was saponified in 5 ml NaOH (10%) and 25 ml EtOH. After stirring at room temp. overnight the mixture was poured into cold H_2O and unreacted ester removed by extracting with Et_2O. The acidified aq. phase was extracted with CH_2Cl_2. Removal of the solvent gave the crude acid (110 mg) which was recrystallized from Et_2O–hexane. Compounds **8–12**, **19–24** and **31–36** were prepared by the same procedure. Analytical and physical data are summarized in Tables 1, 2 and 3.

Ethyl 4-bromo-3-methyl-2-butenoate. A solution of ββ-dimethylethylacrylate (55.0 g, 0.43 mole), *N*-bromosuccinimide (82.4 g, 0.46 mole) and benzoyl peroxide (3.9 g) in 150 ml CCl_4 was refluxed for 2 hr. After cooling and filtering, the soln was successively washed with satd soln of NaCl and H_2O. The solvent was evapd and the residue distilled *in vacuo*. The bromo ester (40.1 g, 45%) boiled at 65°/3 mm Hg.

Ethyl ββ-dimethylacrylyl triphenylphosphonium bromide. Drops of a soln of ethyl 4-bromo-3-methyl-2-butenoate (40.1 g, 0.2 mole) were added to a soln of triphenylphosphine (61.0 g, 0.23 mole) in dry C_6H_6 (150 ml). The mixture was stirred for an additional 12 hr and the precipitated phosphonium salt (75 g, 82%) was filtered and washed with C_6H_6–hexane (mp 161–64°).

Ethyl 3-methyl-5-σ-methoxyphenylpenta-2,4-dienoate (**25**) *and* (**28**). A soln of ethyl ββ-dimethylacrylyl triphenylphosphonium bromide (7.1 g) in EtOH (50 ml) was added gradually to a cooled and stirred soln of sodium ethoxide [from Na (0.25 g)] in EtOH (50 ml)]. The red soln was stirred for an additional 15 min., and then σ-methoxybenzaldehyde (1.4 g) in EtOH (50 ml) was slowly added under a stream of N_2. The suspension was stirred at room temp. for 12 hr, then poured into ice H_2O (200 ml) and extracted with CH_2Cl_2 (4 × 75 ml). The organic extracts were combined, washed with satd NaCl soln, dried and evaporated *in vacuo*. The residue was triturated in warm hexane and the solvent evaporated. The product was analyzed by NMR and showed a proportion of approximately 35% of the Δ^2-*cis*,Δ^4-*trans* isomer and 43% of the Δ^2-*trans*,Δ^4-*trans* isomer. The isomeric esters were isolated by the 'dry column' technique using C_6H_6–hexane (6:4). Compounds **26–30** were prepared by this method, their analytical and physical data are summarized in Tables 3 and 4.

Bioassay I. Lettuce seed germination [*24*]. 50 Lettuce seeds *Lactuca sariva* cv Grand Rapids were placed on one sheet of Whatman No. 1 filter paper (50 mm i.d.) soaked with 1 ml of test soln, and allowed to germinate under illumination (GRO-LUX light, 180 W, at 1 m). The number of seedlings after 24 hr was counted. H_2O and ABA controls were always included, in H_2O the percent of germination reached 95–100%. All ABA-analogs were tested at concentrations ranging from 10^{-4} M–10^{-7} M (Table 5).

Bioassay II. Chlorophyll retention [25]. Mature and fully expanded leaves of *Tropaeolum majus* L. were used for the bioassay. The leaves were thoroughly washed in running H_2O, then sterilized for 20 secs in a 1% commercial sodium hypochlorite soln and washed several times in sterile H_2O. Discs (6 mm) were floated in sterile petri dishes containing 4 ml sterile soln of the test compounds (10^{-4} M–10^{-7} M). After 3 days in darkness at 25° the chlorophyll was extracted into dimethylformamide (48 hr at 2–4° in the dark) and estimated by the absorbance at 665 nm. Each treatment included 6 replicates (Table 6).

Bioassay III. Wheat coleoptile elongation. This bioassay was similar to that described by Nitsch and Nitsch [26] and by Hancock *et al.* [27]. Three 10 mm coleoptile sections were placed in a small vial with 0.75 ml Pi–citrate buffer pH 5.7 + 2% sucrose. The vials were rotated (1 rpm) for 22 hr at 27°, in the dark; control sections usually grew to 18–20 mm. All compounds were bioassayed in concentrations 10^{-4} M–10^{-7} M. (Table 7).

Bioassay IV. Transpiration test. 10-day-old barley leaves grown on vermiculite irrigated with half-strength Hoagland soln under natural light, were cut 8 cm below the tips and two of them were transferred to 20 mm^3 vials containing the test soln. Each vial was covered with parafilm which eliminated H_2O loss, except by transpiration. The transpiration rate was measured as H_2O loss per vial, during 24 hr under illumination. Each compound was tested in concentrations from 10^{-4} M–10^{-7} M. 5 replicates were used for each conc. (Table 8).

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