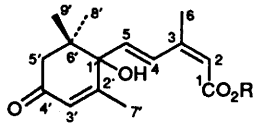
Enantioselective Reduction of Racemic Abscisic Acid by *Aspergillus niger* Cultures

Alberto Arnone, Rosanna Cardillo, Gianluca Nasini and Orso Vajna de Pava
Dipartimento di Chimica del Politecnico, Centro del C.N.R. per le Sostanze Organiche Naturali, Politecnico di Milano, Piazza Leonardo da Vinci 32, 20133 Milano, Italy

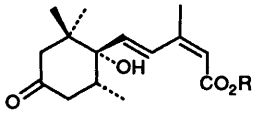
Biotransformation of racemic abscisic acid [(±)-ABA] **1a** with cultures of *Aspergillus niger* gives rise to the enantioselective reduction of the (*S*)-enantiomer to afford the corresponding (1′*S*,2′*R*)-(−)-2′,3′-dihydro-ABA acid **2a** in high enantiomeric excess (e.e.). The structure, stereochemistry, and preferred conformation of compound **2a** have been elucidated on the basis of NMR evidence.

Abscisic acid **1a** is a monocyclic sesquiterpene of universal occurrence in higher plants, isolated from a variety of species, and active as a growth and development regulator.[1] Only the natural (*S*)-isomer is active in stomatal closure;[2] both enantiomers, on the other hand, are active as germination and growth inhibitors.[2] Natural (*S*)-ABA was also isolated from phytopathogenic fungi such as *Cercospora rosicola*[3] and *Botrytis cinerea*.[4]

Recently, it was reported that cell cultures of *Bromus inermis*, when fed with racemic ABA **1a**, metabolize the (*S*)-epimer more rapidly than the unnatural (*R*)-epimer.[5] This fact led us to investigate whether a similar behaviour could be observed for (±)-ABA **1a** by the action of fungal strains.



1a; R = H
1b; R = Me



2a; R = H
2b; R = Me

In this paper we report on the enantioselective reduction of (±)-ABA **1a** by *Aspergillus niger* and on the structure elucidation of the resulting (1′*S*,2′*R*)-(−)-2′,3′-dihydro-ABA **2a**. When submitted for 72 h to a culture of *A. niger* (strain IPV 283) grown in a liquid medium (malt–peptone–glucose), (±)-ABA **1a** afforded a crude mixture containing unchanged (*R*)-ABA and (1′*S*,2′*R*)-(−)-2′,3′-dihydro-ABA **2a**, $[\alpha]_D$ −24.4° in *ca.* 1:1 ratio (72% yield). The structure of compound **2a** and of its methyl ester **2b** were established by ^1H and ^{13}C NMR studies (see later).

The course of the biotransformation was followed by HPLC analysis carried out on the methyl esters of the reacting components, by using a chiral, cellulose-based column.[6] Although the chromatogram of the products shown in Fig. 1(c) contained only two peaks, attributable to ester **2b** and to the methyl ester (*R*)-**1b** of the unchanged (*R*)-ABA, it cannot be excluded that isomers of compound **2b** might have R_f-values analogous to those exhibited by the above compounds. Thus, we have submitted to the same bioconversion conditions pure (*S*)- and (*R*)-ABA epimers, obtained from *Cercospora rosicola*[3] and from the above cited experiment, respectively. No notable transformation was detected for (*R*)-ABA, whereas from (*S*)-ABA it was isolated a compound possessing $[\alpha]_D$ −25.4°, and R_f and ^1H NMR data identical with those exhibited by compound **2a**.

(1′*S*,2′*R*)-(−)-Dihydro-ABA **2a** was isolated as a white solid, m.p. 85 °C; the IR spectrum showed absorptions at 3430 cm^{-1} (OH), and 1690 and 1935 cm^{-1} (CO), and the UV spectrum absorptions at 255 nm (ε 14 000), in agreement with the presence of an α,β,γ,δ unsaturated ester moiety.[7] It analysed for $C_{15}H_{22}O_4$ (M^+, 266) and differs in molecular weight by two mass units from compound **1a**. A comparison of the ^1H NMR data of compounds **1a** and **2a** (Table 1) revealed a close similarity between the two compounds, the only significant difference being the replacement of the C(2′)Me=C(3′)H moiety of compound **1a** by a C(2′)HMe–C(3′)H_2 grouping in compound **2a**. The presence of a CO_2H group in product **2a** was confirmed by the formation of the methyl ester **2b** upon treatment of acid **2a** with CH_2N_2. An evaluation of the coupling constants of the protons of the cyclohexanone ring and the NOE experiments carried out on ester **2b** indicate that this ring preferentially adopts the chair-like conformation depicted in Fig. 2 in which the C-1′ hydroxy group is axially disposed. In fact, the vicinal coupling constant of 12.5 Hz between 2′-H, assumed as β, and 3′-H^α (δ 2.47), and the W-type long-range coupling constant of 1.0 Hz between 5′-H^α (δ 2.88) and 9′-H_3^β (δ 0.96) point to a *trans* diaxial configuration for each pair of protons, while the W-type long-range coupling constant of 2.2 Hz between 3′-H^β (δ 2.22) and 5′-H^β (δ 1.92) indicates that these protons are diequatorially disposed. The enhancements observed for the axially disposed 3′-H^α (1.5%) and 5′-H^α (1%), and for the equatorially disposed 7′-H_3^α (1.1%) and 8′-H_3^α (0.5%) upon irradiation of the proton of the C-1′α hydroxy group (see Fig. 2 and Experimental section) require that all these protons are on the same α-side of the molecule.

The chirality of the newly formed C-2′ and thus the absolute configuration of compound **2a**, *i.e.* (1′*S*,2′*R*), were consequently determined. Also, the ^{13}C NMR spectrum of acid **2a** (see Experimental section) is in agreement with the proposed structure. Literature data on biotransformation of sesquiterpenes[8] and iononic ABA-analogues[9] with *Aspergillus niger* report mainly on oxidative reactions with formation of hydroxy and oxo derivatives. Only for (*R*)- and (*S*)-carvone,[10] was there observed a reduction of the endocyclic double bond and then reduction of the conjugated ketonic group.

In our case the bioagent *A. niger* showed multiple selectivity.

Table 1. ^{1}H NMR chemical shifts (δ_H) and ^{1}H–^{1}H coupling constants (J/Hz) for compounds **1a**, **2a** and **2b** in $CDCl_3$.

Proton	1a	2a	2b	H,H	J(H,H) 1a	J(H,H) 2a	J(H,H) 2b
2	5.77	5.77	5.74 (5.71)[a]	2,4	0.7	0.7	0.7
4	6.17	6.17	6.12 (6.21)	2,5	0.9	0.8	0.9
5	7.81	7.81	7.84 (7.84)	2,6	1.3	1.3	1.4
6	2.05	2.06	2.03 (2.05)	4,5	16.1	16.3	16.3
2′β		2.36	2.33 (2.39)	2′β,3′α		12.0	12.5
3′α	5.97	2.48	2.47 (2.50)	2′β,3′β		3.7	4.1
3′β		2.24	2.22 (2.16)	2′β,7′		6.2	6.4
5′α	2.49	2.88	2.88 (2.92)	3′α,3′β		12.5	13.1
5′β	2.30	1.95	1.92 (1.86)	3′α,5′α		1.1	1.0
7′	1.93	0.90	0.89 (0.95)	3′β,5′β	1.1	2.1	2.2
8′	1.04	0.98	0.98 (1.01)	3′,7′	1.4		
9′	1.12	0.96	0.96 (0.98)	5′α,5′β	17.0	13.6	13.5
1-OR	3.60	5.50	3.72 (3.68)	5′α,9′	1.0	0.9	1.0
1′-OH	3.60	5.50	1.94 (3.53)				

[a] Values in parentheses are chemical shifts in $CDCl_3$–[$^2H_6$]acetone (1:1).

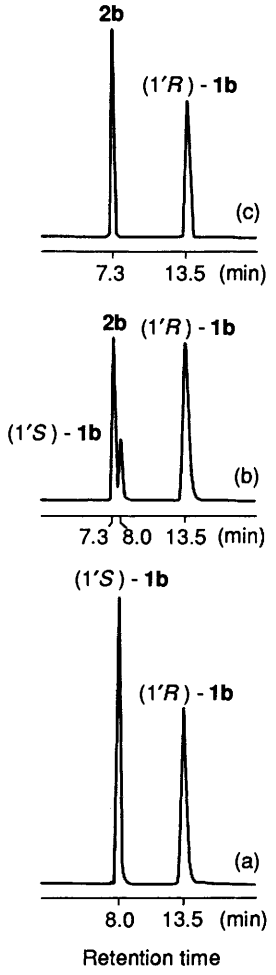


Fig. 1. HPLC chromatograms of (a) the starting (±)-ABA methyl ester **1b**, (b) the reacting mixture after 2 days and (c) products.

It reacted with the (*S*)-ABA enantiomer **1a** (e.e. >95%), reducing regioselectively the endocyclic double bond to afford the (1′*S*,2′*R*)-2′,3′-dihydro-ABA diastereoisomer **2a** (d.e. >95%). A synthetic (±)-2′,3′-dihydro-ABA, active as a growth inhibitor on rice seedlings, has also been reported,[11] the relative stereochemistry of the 2′- and 3′-alkyl groups being *cis*, in opposition to the *trans* stereochemistry observed in compound **2a**. Work is in progress to extend the method to other micro-organisms and substrates.

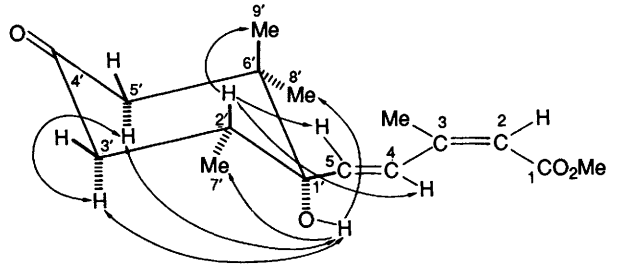


Fig. 2. Selected NOE enhancements and preferred conformation for dihydroabscisic acid methyl ester **2b**.

Experimental

M.p.s were measured on a Kofler apparatus and are uncorrected. UV spectra were measured for solutions in 95% EtOH on a JASCO Uvidec-510 spectrophotometer. IR spectra were recorded with a Perkin-Elmer 177 instrument. TLC and PLC were performed with Merck RP-18 silica gel. Optical rotations were measured on a JASCO DIP-181 polarimeter. HPLC analyses for compounds (*R*)- and (*S*)-**1b**, and ester **2b** were performed on a JASCO Twincle apparatus using a Daicel Chiralcel OD 0.45 × 25 cm column with hexane–Pr^{i}OH (9:1) as eluant at a nominal flow rate of 1 cm^3 min^{-1}. The retention time (t_R) observed for esters **2b**, (*S*)- and (*R*)-**1b** were 7.3, 8.0 and 13.5 min, respectively. Mass spectra were taken on a VG-ZAB2 instrument at 70 eV. ^{1}H NMR spectra were recorded on a Bruker CPX-300 (300.13 MHz) spectrometer, and ^{13}C NMR spectra on a Bruker AC 250L (69.2 MHz) instrument. Chemical shifts (δ) are in ppm from $SiMe_4$ as internal standard. NOE values reported in the text have only qualitative significance.

Culture Conditions and Procedures for Microbial Reduction.—The strain *Aspergillus niger* (IPV 283) was maintained on MPGA (malt, peptone, glucose, agar 20:4:30:15 g dm^{-3}) slants at 24 °C, and subcultured in 18 shaken Erlenmeyer flasks containing a liquid medium MPG (50 cm^3) for 48 h at 24 °C. Racemic ABA (10 mg per flask), as a solution in dimethyl sulphoxide (0.1 cm^3), was added to the growing cultures, and

the incubation was continued for 72 h in shaken flasks at 24 °C.

Isolation of the Biotransformation Products.—The culture filtrates, separated from the mycelium, were extracted with EtOAc, and the extracts were dried (Na_2SO_4) and evaporated to give a mixture of crude metabolites. The mixture was then chromatographed on a column of RP-18 silica gel with acetone–water (1.5:1 v/v) to yield unchanged (*R*)-(−)-ABA (70 mg) and (1′*S*,2′*R*)-(−)-2′,3′-dihydro-ABA **2a** (60 mg) (72% yield; R_f 0.45 and 0.36, respectively).

(1′S,2′R)-2′,3′-*Dihydro-ABA* **2a**. This *compound* was obtained as a white solid; $[\alpha]_D$ −24.4° (*c* 0.10, EtOH); e.e. >95% (Found: C, 68.5; H, 6.6. $C_{15}H_{18}O_4$ requires C, 68.68; H, 6.92%); *m/z* (EI), 266 (M^+), 248 ($M^+ - 18$), 232, 192, 165 and 125 (base peak); $\delta_C(CDCl_3)$ 211.49 (s, C-4′), 170.74 (s, C-1), 151.67 (s, C-3), 140.58, 127.71 and 117.13 (d, C-2, -4, and/or -5), 78.04 (s, C-1′), 51.43 and 45.04 (t, C-3′ and/or -5′), 43.04 (s, C-6′), 36.71 (d, C-2′) and 24.69, 24.62, 21.54 and 16.10 (q, C-6, -7′, -8′ and -9′). ^{1}H NMR data are reported in Table 1. A compound which exhibited a ^{1}H NMR spectrum identical with acid **2a** and $[\alpha]_D$ −25.4° was obtained in enantiomerically pure form by addition of (*S*)-(+)-ABA (10 mg) to the growing cultures (50 cm^3) of *A. niger* under the above described conditions whereas the (*R*)-(−)-ABA, isolated previously, was recovered unchanged.

(1′S,2′R)-2′,3′-*Dihydro-ABA Methyl Ester* **2b**.—Compound **2a** (10 mg) was dissolved in CH_2Cl_2 (5 cm^3) and treated with CH_2N_2–Et_2O at 0 °C for ten min. Evaporation of the solvent gave ester **2b** as a solid, m.p. 75 °C; λ_{max}(EtOH) 260 nm (ε 19 300); ν_{max}(KBr) 3450 (OH), 1720 (CO ester) and 1690 cm^{-1} (CO); *m/z* (EI) 280 (M^+), 262, 249, 196 and 192 (base peak); ^{1}H NMR data are reported in Table 1. Some connectivities established by NOE difference experiments in $CDCl_3$–[$^2H_6$]-acetone (1:1) are as follows:

Proton irradiated	Proton affected (%)
1′-OH	3′-H$^\alpha$ (1.5), 4-H (1), 5-H (8), 5′-H$^\alpha$ (1), 7′-H_3 (1), 8′-H_3 (0.5)
2′-H$^\beta$	3′-H$^\beta$ (8.5), 4-H (4.5), 5-H (1.5), 7′-H_3 (1.5), 9′-H_3 (1.5)
3′-H$^\alpha$	1′-OH (2), 3′-H$^\beta$ (14), 5′-H_α (1.5), 7′-H_3 (1)
3′-H$^\beta$	2′-H$^\beta$ (8.5), 3′-H$^\alpha$ (12), 7′-H_3 (0.5)
5′-H$^\alpha$	3′-H$^\alpha$ (1.5), 5′-H$^\beta$ (15), 8′-H_3 (0.5)
5′-H$^\beta$	5′-H$^\alpha$ (15), 8′-H_3 (0.5), 9′-H_3 (0.5)
6-H_3	2-H (11.5), 4-H (11), 5-H (1)

(R)-(−)-*ABA* **1a**. This *compound* was crystallized from $CHCl_3$–hexane as a white solid, m.p. 160 °C; $[\alpha]_D$ −350 °C (*c* 0.5, EtOH) (Found: C, 68.1; H, 7.5. $C_{15}H_{20}O_4$ requires C, 68.16; H, 7.63%); λ_{max}(EtOH) 250 nm (ε 17 000); *m/z* (EI), 264 (M^+), 246 ($M^+ - H_2O$), 190, 162, 134 and 111.

(R)-(−)-*ABA methyl ester* **1b**. (*R*)-(−)-ABA **1a** (10 mg) was dissolved in CH_2Cl_2 (5 cm^3) and treated with CH_2N_2–Et_2O at 0 °C. Evaporation of the solvent gave the ester **1b** as a white solid, m.p. 87 °C; ν_{max}(KBr) 3400 (OH), 1710 (CO ester) and 1650 (CO) cm^{-1}; *m/z* (EI), 278 (M^+), 260 ($M^+ - H_2O$), 245, 190, 162, 125 and 107.

Acknowledgements

This work was supported by Consiglio Nazionale delle Ricerche (CNR) Roma, Progetto finalizzato «Chimica Fine II».

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Paper 0/02566F
Received 8*th June* 1990
Accepted 12*th July* 1990