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Note

Optical resolution of abscisic acid metabolites using an ovomucoid-conjugated high-performance liquid chromatographic column

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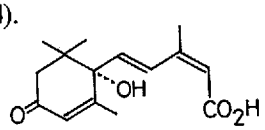
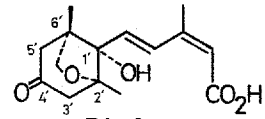
Abscisic acid (ABA, **1**) plays important roles in many aspects of plant growth and development[1]. Studies on the metabolism of ABA involve particularly difficult analytical problems. As with all studies of metabolism using racemic substrates, establishing the optical purity of the products is very important. Especially for trace components such as plant hormones and their metabolites, this can only be done with considerable difficulty.

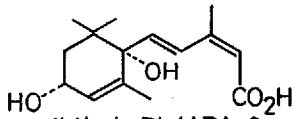
Recently we achieved the direct optical resolution of racemic ABA by high-performance liquid chromatography (HPLC) on an ovomucoid-conjugated column, ULTRON ES-OVM[2]. Very little is known about the optical resolution of the metabolites of ABA. We report here the direct optical resolution of the metabolites of ABA, phaseic acid (PA, **2**) and the 1′,4′-diols of abscisic acid (1′,4′-*cis*- and -*trans*-diol-ABA, **3** and **4**).



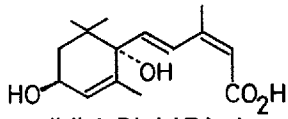
ABA, 1



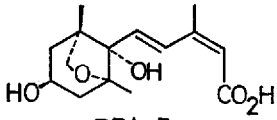
PA, 2



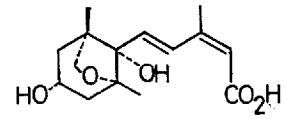
1′,4′-cis-DiolABA, 3



1′,4′-t-Diol ABA, 4



DPA, 5



epi-DPA, 6

0021-9673/90/$03.50

EXPERIMENTAL

Apparatus

A Shimadzu LC-5A instrument equipped with an SPD-2A variable-wavelength UV Monitor was used. A stainless-steel column (150 × 4.6 mm I.D.) was packed with ovomucoid-conjugated aminopropylsilica gel (5 μm), now available as ULTRON ES-OVM (Shinwakako, Kyoto, Japan).

Chemicals

(1′*S*, 2′*R*, 6′*R*)-PA (natural form) and (1′*R*, 2′*S*, 6′*S*)-PA (unnatural form) were kindly supplied by Dr. T. Kitahara. Racemic ABA was bought from Wako (Osaka, Japan). (+)-(*S*)-ABA was provided by Dr. N. Hirai. The 1′,4′-diols of ABA were synthesized as described previously[3]. Dihydrophaseic and *epi*-dihydrophaseic acid (DPA, **5**, and *epi*-DPA, **6**) were prepared by the method of Hirai and Koshimizu[4]. All other chemicals were of analytical-reagent grade.

RESULTS AND DISCUSSION

Our previous studies[2] indicated that the resolution of ABA on the ovomucoid-conjugated column could be regulated by varying the pH and hydrophobicity of the mobile phase. This result led to the conclusion that ABA was best resolved in the use of 2% 2-propanol in 20 m*M* potassium phosphate buffer (pH 3.50) as the mobile phase. Under these conditions, we attempted to separate the enantiomers of ABA metabolites (**2**–**6**). The chromatographic results are summarized in Table I and typical chromatograms are shown in Figs. 1 and 2. In these separations, the retention of natural isomers was always longer than that of unnatural isomers, showing that the affinity between the ovomucoid and natural isomers may be more stable. The chiral ovomucoid-conjugated column (ULTRON ES-OVM) showed beter enantioselectivity for the 1′,4′-diols of ABA (**3** and **4**) than that for ABA. It is suggested that the 4′-hydroxyl group of the diols contributes more significantly than the 4′-keto group of ABA to the interaction with ovomucoid.

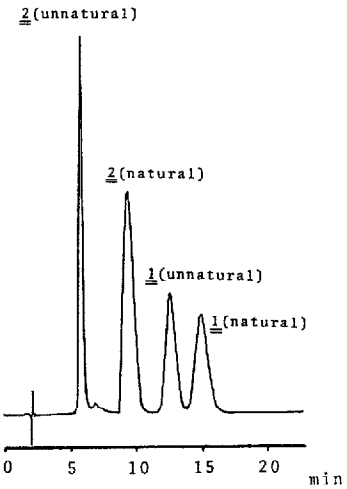
An excellent separation factor (α = 1.97) was obtained in the enantiomeric

TABLE I

DIRECT ENANTIOMERIC SEPARATION OF ABA AND ITS DERIVATIVES BY HPLC WITH ULTRON ES-OVM

A 2% 2-propanol–20 m*M* potassium phosphate buffer (pH 3.50) was used as the mobile phase at 1.0 ml/min at ambient temperature with UV detection (254 nm, 0.04 a.u.f.s.). ABA was best resolved under these conditions.

Compound	k'_1	k'_2	α
ABA	10.00	12.20	1.22
PA	3.53	6.94	1.97
1′,4′-*cis*-Diol-ABA	10.90	14.99	1.38
1′,4′-*trans*-Diol-ABA	4.40	6.14	1.40
DPA	1.74	–	1.00
epi-DPA	2.45	–	1.00



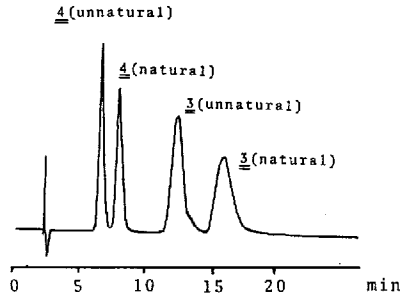


Fig. 1. Separation of the enantiomers of ABA (**1**) and PA (**2**). Chromatographic conditions as in Table I.

Fig. 2. Separation of the enantiomers of 1′,4′-*cis*- and *trans*-diol-ABA (**3** and **4**).

separation of PA. This enantioselectivity may be attributed to the fact that tetrahydrofuran bridge containing ether oxygen is available for hydrogen bonding interactions with the chiral stationary phase. This column has no enantioselectivity for DPA (**5**) and *epi*-DPA (**6**) which are very polar metabolites of ABA. The lack of enantioselectivity may be ascribed to their smaller hydrophobicity, because ABA, 1′,4′-diols of ABA and PA were separated to a considerable extent. Previously we showed[2] that hydrophobic interactions may play important roles in the retention of ABA, but that they are not essential for the resolution. This experiment shows that the ovomucoid column has advantages for the resolution of compounds with suitable hydrophobicity.

This is the first report on the optical resolution of ABA-related compounds. PA is the major catabolite of ABA which is subsequently reduced to DPA and *epi*-DPA[1]. 1′,4′-*cis*-Diol-ABA is assumed to be a natural catabolite[5]. 1′,4′-*trans*-Diol-ABA can act as a precursor of ABA rather than being a catabolite[3,6–8].

Therefore, we believe that the results of this study will be useful when examining the enantioselectivity of the enzymes which catalyse the metabolism of ABA.

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