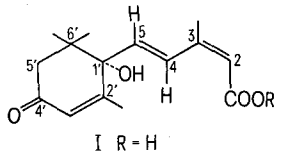
^{13}C chemical shifts (δ) of (+)-ABA

Mult.	δ	Carbon
q	19.1	Me – 2′
q	21.4	Me – 3
q	23.1	2Me – 6′
q	24.3	
s	41.7	C – 6′
t	49.7	C – 5′
s	79.9	C – 1′
d	118.1	C – 2
d	127.0	C – 3′
d	128.3	C – 4
d	136.8	C – 5
s	151.4	C – 2′
s	163.0	C – 3
s	170.9	C – 1
s	198.3	C – 4′

which is frequently found on *Rosa* sp.[6], produces (+)-ABA (6 mg/100 ml maximum) when grown on a potato-agar (PA) medium, at pH 6.5–6.8, 24 °C in the light for 30–40 days. Other culture media, such as yeast-glucose-agar (2% yeast and 20% glucose), malt-agar, oatmeal-agar, are also effective. On stationary potato broth, the average amount of ABA decreases to 2 mg/100ml, whereas no ABA was found when the fungus was grown on Sabouraud maltose agar, GPA, (glucose 30%, peptone 3%, agar), Czapek agar or nutrient agar. Production is much higher than the average amount of ABA in plants[2,7]. The compound was obtained from cultures by direct extraction with AcOEt and chromatography through silica gel with hexane-AcOEt mixtures as eluent.



I R = H
II R = Me

Pure (+)-ABA, $[\alpha]_D^{20} = +384°$ (EtOH, c = 0.23) (Milborrow[8] + 430°) was identified from m.p., UV, IR, NMR and mass spectra of the acid and of the methyl ester (**II**), which appeared identical to those given in the literature[2], and by comparison with a commercial sample of (±)-ABA.

The table lists the ^{13}C NMR resonances ($CDCl_3$, 23.6 MHz) for **I**, which have not been reported so far, and may be useful for biosynthetic studies.

The assignment of the carbons is based on analysis of the undecoupled and off-resonance spectra. In particular, C-3′, Me-2′, C-4, C-5, C-2 and Me-3, have been assigned by selective decoupling for the corresponding ^1H signals[9] and C-2′, C-3 have been distinguished by comparison with data for (+)-S-dehydrovomifoliol[10].

If the production of (+)-ABA, as well as that of auxins by phylloplane fungi[11], occurs also in vitro, this would suggest that fungi may play a role in growth regulation within the ecosystem.

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The use of benzylpenicillinacylase from *Escherichia coli* in the resolution of some racemic β-, γ-, δ- and ε-amino-acids

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Summary. The enzymatic hydrolysis of N-phenylacetyl derivatives of racemic amino-acids having the chiral centre removed from the usual α-position is examined. The reaction is found to have different degrees of stereoselectivity. In the case of β-amino-acids and of γ-aminovaleric acid, both enantiomers can be obtained in good yields and high optical purity. S-directed stereochemical preference was found for all the substrates examined.

Preferential hydrolysis of N-acyl derivatives of a variety of L α-amino-acids by enzymes has been exploited for the resolution of racemic mixtures, as well as for the assignment of the absolute configuration.

To our knowledge, no data are available concerning enzymatic hydrolyses of N-acyl derivatives of amino-acids having the chiral centre progressively removed from its usual position α to the carboxyl group. In an effort to explore the synthetic utility, the limitations as well as the rate and the stereoselectivity of the hydrolyses of these substrates, we have examined the enzymatic hydrolysis of the N-phenylacetyl derivatives (N-PA-derivatives) of amino-acids **1–7**.

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	Compound	R	n	
R	**1**	Me	1	CH_2–NH_2
CH–NH_2	**2**	Me	2	CH–CH_3
$(CH_2)_n$	**3**	Me	3	COOH
COOH	**4**	Me	4	**7**
	5	Et	1	
	6	Ph	1	

The choice of the substrates was restricted to amino-acids having known absolute configuration. Benzyl-penicillinacylase (BPA) from *Escherichia coli* A.T.C.C. 9637 was selected as the deacylating enzyme. It is well-established that BPA shows a high degree of stereo-selectivity in hydrolyzing N-PA-α-amino-acids[2–5] together with a low degree of substrate specificity[2, 3, 5–7].

Material and methods. N-PA-derivatives were synthesized by treating the corresponding amino-acid with phenyl-acetyl chloride in dilute aqueous sodium hydroxide and were crystallized from AcOEt-hexane. The BPA used showed a specific activity of 2000 units/mg[8]; details of the purification method adopted by us will be reported elsewhere.

Hydrolysis experiments were accomplished as follows. 10 mmoles of N-PA-derivative in 200 ml of water containing 6.6 mmoles of $CaCO_3$ and 6.0 mg of BPA were briefly agitated to attain complete solution of the substrate and incubated at 37 °C. The incubation was continued until the amount of the released phenylacetic acid accounted for 50% hydrolysis of the starting racemic substrate and was then stopped. The time course of production of phenylacetic acid was followed by gas-chromatography (internal standard methyl benzoate) by esterifying with diazomethane aliquots at suitable intervals. Oxalic acid (6.6 mmoles) was added to the reaction mixtures. N-PA-derivatives, together with the phenyl-acetic acid, were then separated by continuous extraction with ether. The aqueous phases were filtered. Evaporation under vacuum, and crystallization of the residues, gave the amino-acids released by the enzyme.

The ethereal phases were evaporated to dryness and the residues subjected to column chromatography on silica gel (30 g of silica per g of residue). Elution with benzene: AcOEt (8:2) gave phenylacetic acid. On subsequent elution with AcOEt, the unreacted N-PA-derivatives were recovered in practically quantitative yield. The N-PA-derivatives were hydrolyzed by refluxing for 24 h in 10 ml of 2 N hydrochloric acid. Phenylacetic acid was separated by washing the acidic aqueous solutions with ether. Evaporation of the aqueous phases and treating of the residues with Amberlite IR-4B gave the amino-acids.

Results and discussion. The data in the table show that the enzymatic hydrolysis of the N-PA-derivatives with BPA is a valuable method for the production of optically active β-amino-acids **1**, **5**, **6** and for the γ-aminovaleric acid **2**. Indeed both enantiomers can be obtained in good yields with a high degree of optical purity. These advantages are rarely experienced when resolution based upon the crystallization of diastereomeric derivatives is used. In the case of the δ-amino-acid **3** and of the ε-amino-

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Substrates examined and results of the hydrolysis experiments

Compound	M.p. of the starting racemic N-PA-derivative	Incubation time (h)[a]	$[\alpha]_D$ of the recovered N-PA- derivative[b]	Amino-acid released by the enzyme[c]		Amino-acid obtained by HCl hydrolysis of the recovered N-PA-derivative[c]		$[\alpha]_D$ Maximum literature value (absolute configuration)	
				$[\alpha]_D$	Yield[d]	$[\alpha]_D$	Yield[d]		
1 β-Aminobutyric acid	107–108 °C	9	+13.0° (c=1)	+34.8° (c=1)	78%	−36.0° (c=1)	75%	+35°	(S)[10]
2 γ-Aminovaleric acid	118–119 °C	19	+ 6.8° (c=4)	−12.5° (c=1)	80%	+13.0° (c=1)	75%	+13.9°[10]	(R)[9]
3 δ-Aminocaproic acid	90– 92 °C	30	+ 2.1° (c=3)	− 2.0° (c=4)	72%	+ 2.1° (c=4)	75%	+ 5°	(R)[9]
4 ε-Aminoheptanoic-acid	100–102 °C	35	+ 3.5° (c=4)	− 0.7° (c=5)	76%	+ 0.9° (c=5)	78%	+ 2.4°	(R)[12]
5 β-Aminovaleric acid	96– 98 °C	29	+21.0° (c=5)	+38.0° (c=2)	75%	−38.7° (c=2)	72%	+38.5°	(S)[12]
6 β-Phenyl-β-alanine	134–136 °C	45	−66.0° (c=5)	+ 7.5° (c=1.5)	88%	− 7.4° (c=1.5)	70%	− 6.9°	(R)[13]
7 α-Methyl-β-alanine	110–111 °C	1.2	− 1.8° (c=10)	+ 1.1° (c=7)	74%	− 0.5° (c=10)	75%	−15.3°	(R)[14]

See text for full experimental details. All $[\alpha]_D$ determinations performed at 20 °C; solvent is absolute EtOH for the N-PA-derivatives and water for the amino-acids. All the products obtained by chemical methods or by enzymatic hydrolysis showed correct elemental analysis and spectroscopic data. [a]Corresponding to 50% hydrolysis of the starting racemic substrate. [b]These compounds, chromatographically pure, were not crystallized. [c]Optical rotations and yields are referred to the amino-acids obtained after one crystallization. Compounds **1–5** and **7** were crystallized from MeOH – Et_2O; compound **6** was crystallized from H_2O – EtOH. [d]R- or S-enantiomer in the starting racemic mixture = 100%.

acid **4** (in which the asymmetric centre is removed even farther from the carboxyl), the difference between the rates of hydrolysis of the 2 enantiomers is not great enough to allow an optical purity useful for preparative purposes to be obtained. A comparison of the specific rotations found with the respective maximum values reported in the literature, shows, in fact, that the optical purity is about 40% for the δ-amino-acid **3** and 35% for the ε-amino-acid **4**. Passing from the γ-amino derivative to the subsequent ones seems then to be critical for the stereoselectivity of the hydrolysis in the conditions adopted.

Examination of the data in the table clearly shows that, for all the substrates examined, BPA preferentially hydrolyzes the S-enantiomers. This stereoselectivity is not lost even in the case of the hydrolysis of the N-PA-derivative of α-methyl-β-alanine **7**, in which the acylamino group is not directly bonded to the chiral centre. A significant consequence of the above observation concerns the possibility of evaluating the opposite assignments[9-11], made via chemical correlations, of the absolute configuration to γ-aminovaleric acid **2**, a compound of current chemical and biochemical interest. The hydrolysis data of the table are in accordance with the configurational assigment R(+) made previously by us[9]; this assignment is reported in the table. The statement made by other authors[10,11] according to which in the series 'alanine, β-aminobutyric acid, γ-aminovaleric acid', the enantiomers possessing the same sign of optical rotation, have the same configuration, finds no support, even on biochemical grounds.

As far as the rates of hydrolysis are concerned, 2 preliminary observations can be made regarding the influence of the distance of the amido group from the carboxylic group and the substitutions at the asymmetric carbon atom. The hydrolysis rates seem to be more influenced by the hindrance of the substituents at the asymmetric carbon atom than by the progressive removal of the same carbon atom from the carboxylic group. This effect becomes evident if the incubation times for compounds **1**–**4** are compared with those for compounds **1** and **6** and for compounds **1** and **5** (table).

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Methionine metabolism in apple tissue

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Summary. A metabolic intermediate isolated from apple tissue fed either methionine or 5′-methylthioadenosine has been tentatively identified as a methionine-pyridoxal Schiff base. The formation of this compound is discussed in relation to ethylene biosynthesis.

Methionine has been established as a precursor of ethylene in a number of fruit and vegetative tissues, but it is not the immediate precursor[2]. In apple tissue recent evidence suggests that methionine is converted into S-adenosylmethionine prior to its conversion to ethylene[3-5], that pyridoxal phosphate is involved as coenzyme[4-6], and that 5′-methylthioadenosine is a fragment nucleoside product[4,5,7]. The formation of 5′-methylthioadenosine is consistent with the observation that no volatile sulfur compounds are recovered during the conversion of methionine to ethylene[8,9].

Although methionine is an excellent precursor of ethylene in fruit tissue, there is a lag period of about 40 min before a steady rate of ethylene production from methionine is observed[10]. It has beeen noticed that apple tissue produced little ethylene in a nitrogen atmosphere, but that ethylene production was greatly stimulated upon transfer to air[11]. These results suggest that an intermediate(s) is (are) involved in the conversion of methionine to ethylene which accumulates in the presence of nitrogen and is degraded rapidly in air. Although there has been speculation as to the possible intermediate(s) in ethylene synthesis from methionine[2], there have been no reports concerning isolation and identification of the intermediate(s). This paper describes the formation of methionine-pyridoxal Schiff base from either methionine-[Me-^{14}C] or 5′-methylthioadenosine-[Me-^{14}C] fed to apple tissue.

Materials and methods. Apples (cv. Golden Delicious) were purchased from a local market. S-Adenosyl-L-methionine-[Me-^{14}C] was purchased from New England Nuclear. 5′-Methylthioadenosine-[Me-^{14}C] was prepared by hydrolysis of S-adenosyl-L-methionine-[Me-^{14}C] for 20 min at 100 °C in dilute HOAc, pH 4.0[12]. Verification that only labelled 5′-methylthioadenosine was present in the hydrolyzate was done by paper co-chromatography and co-electrophoresis with authentic sample as described previously[5]. Methionine-[Me-^{14}C] was a product of Amersham/Searle. Apple plugs (1 cm in diameter and 2 cm in length) were cut from the fruit with a cork borer and razor blade. The

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