Phytochemistry, 1979. Vol. 18. pp. 105–110. © Pergamon Press Ltd. Printed in England. 0031-9422/79/0101-0105 $02.00/0

MUTUALLY EXCLUSIVE OCCURRENCE AND METABOLISM OF TRIGONELLINE AND NICOTINIC ACID ARABINOSIDE IN PLANT CELL CULTURES*

ULRIKE WILLEKE, VOLKER HEEGER, MARIA MEISE, HEINER NEUHANN, INGRID SCHINDELMEISER, KARIN VORDEMFELDE and WOLFGANG BARZ†

Lehrstuhl für Biochemie der Pflanzen der Universität, D-4400 Münster, W. Germany

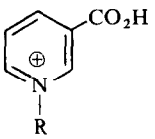
(*Received* 20 *February* 1978)

Key Word Index—Angiospermae; Gymnospermae; cell suspension cultures; trigonelline; nicotinic acid *N*-α-L-arabinoside; chemosystematics.

Abstract—In 50 cell suspension cultures of wide taxonomic origin, formation of trigonelline and nicotinic acid *N*-α-L-arabinoside from nicotinate was strictly alternative. The arabinoside was only found in cell cultures of the subclass Asteridae and in the higher orders of the subclasses Rosidae and Dilleniidae. Degradation of nicotinic acid could only be observed in cell cultures producing the arabinoside. Nicotinic acid degradation does not involve free 6-hydroxynicotinic acid. Cross feeding experiments with both conjugates and measurements of a nicotinic acid *N*-arabinoside: UDP-arabinosyltransferase support the hypothesis that metabolism of these two derivatives in cell cultures may be of chemosystematic value. Finally various discrepancies between plants and cell cultures with respect to nicotinate metabolism and to the natural occurrence of the two conjugates are discussed.

INTRODUCTION

Upon application of nicotinic acid [1–3], nicotinamide [3, 4] or [carbonyl-^{14}C]-NAD [5] to various plant cell suspension cultures, the rapid formation of either trigonelline (**1**) or nicotinic acid *N*-α-L-arabinoside (**2**) has been observed. Both **1** and **2** are formed in large amounts and can be regarded as reservoir forms of nicotinic acid and thus pyridine nucleotides [3, 5].



R = Me, **1** trigonelline
R = Ara, **2** nicotinic acid arabinoside

Trigonelline has long ago been shown to be of widespread occurrence in plants [6–9], and much later in plant cell suspension cultures [1, 3, 10] and in animals [11, 12], whereas **2** has only recently been identified [2, 4] in feeding experiments with various cell suspension cultures. It has also been recognized as a natural constituent in parsley cell suspension cultures [2] while its occurrence in intact higher plants has so far only been preliminarily described [13].

In our previous studies [1–5], cell suspension cultures of 8 plants had been investigated and the formation of either **1** or **2** appeared to be mutually exclusive. Thus cultures of *Petroselinum crispum* and *Daucus carota* (Umbelliferae) as well as those of *Nicotiana tabacum* and *N. glauca* (Solanaceae) formed **2**, whereas cell cultures of *Phaseolys aureaus*, *Cicer arietinum* and *Glycine max* (Leguminosae) and of *Chenopodium rubrum* (Chenopodiaceae) yielded trigonelline. Furthermore, the capacity for nicotinic acid degradation by the cell cultures seemed to be associated with the occurrence of the arabinoside. These results have led to further studies on nicotinic acid conjugation and degradation in a variety of plant cell suspension cultures from both angiosperms and gymnosperms to determine the metabolic relation between **1** and **2**.

RESULTS

Metabolites of nicotinic acid

Batch-propagated cell suspension cultures of altogether 50 plants were investigated and mostly used in late logarithmic growth phase. [Carboxyl-^{14}C]-nicotinic acid (10^{-5} M, 1 μCi) was given under aseptic conditions for 48 hr and $^{14}CO_2$ was collected. In most cases the substrate was rapidly and quantitatively absorbed by the cells. Cell extracts were analysed for distribution of radioactivity in nicotinate metabolites by PC (solvents L_1, L_2) or paper electrophoresis followed by scanning. **1** and **2** were further identified according to previous methods [1–3]. Without exception, the plant cell suspension cultures formed either trigonelline or nicotinic acid if arabinoside. Usually, formation of either **1** or **2** occurred to a very high extent. As an example, Fig. 1 demonstrates the mutually exclusive formation of either **1** (*Dioscorea composita*) or **2** (*Digitalis purpurea*). Considerable care has been taken to demonstrate by dilution analyses the absence of **2** from cell cultures producing

* Part VI in the series "Metabolism of Nicotinic acid in Plant Cell Suspension Cultures". For Part V, see ref. [5].

†To whom correspondence should be addressed.

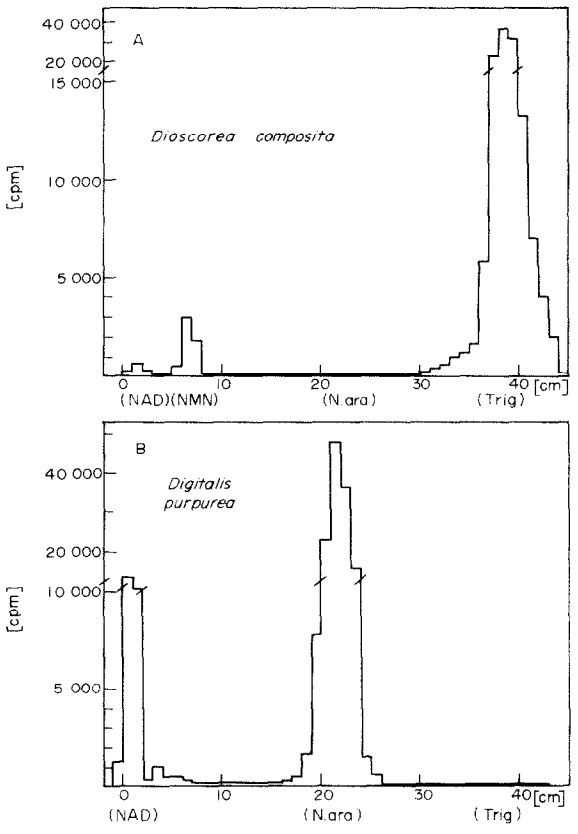


Fig. 1. Distribution of radioactivity in trigonelline (Trig) or nicotinic acid-*N*-α-L-arabinoside (N. ara) in cell extracts of *Dioscorea composita* (A) and *Digitalis purpurea* (B) after application of [carboxyl-^{14}C]-nicotinic acid. Nicotinic acid and nicotinamide migrate much faster and their location on PC is not shown. Chromatograms were developed with 85% *i*-PrOH.

trigonelline and vice versa. In no case could both compounds be isolated from the same culture. Therefore the mutually exclusive formation of **1** or **2** may be characteristic for plant cell cultures. The results of our analyses are summarized in Fig. 2 using the classification of Ehrendorfer [14]. All cell cultures investigated of the subclass Asteridae and those from the highest orders of the Rosidae and Dilleniidae produced the arabinoside while all the other cell suspension cultures of the Dicotyledoneae, the Monocotyledoneae and the Gymnospermae formed trigonelline. In case of *Chenopodium rubrum* (Caryophyllidae) both colourless heterotrophic and green photoautotrophic [15] cell suspension cultures were used. Though these two kinds of cultures greatly differ with respect to energy and carbohydrate metabolism, respiration, lipid composition, pigmentation and photosynthetic activity [16 and unpublished], no difference in the formation of trigonelline as the most prominent metabolite could be observed. Nicotinic acid metabolism to either **1** or **2** in cell suspension cultures, therefore, might be of chemosystematic value.

Endogenous occurrence of trigonelline

1 and **2** should not be regarded as simple detoxification products of exogenously applied nicotinic acid but rather as normal cellular constituents. This has previously been shown by the isolation of the arabinoside from parsley cell suspension cultures grown without nicotinic acid [2] and is further documented by our experiments on trigonelline occurrence in leguminous cell cultures. For the successful isolation of **1**, cell suspension cultures of *Glycine max* (B_5 medium [20]) as well as of *Cicer arietinum* and *Phaseolus aureaus* (PRL-4-C medium [21]) had first to be grown on a nicotinate-free medium to remove any trigonelline from exogenous sources. Attempts to grow *C. arietinum* and *Glycine max* for a longer period of time without nicotinic acid failed because growth steadily decreased and completely stopped after 5–7 transfers into fresh medium. *Phaseolus aureus* cell suspension cultures, however, have been grown without nicotinate for some 20 passages (8–10 days each) without any reduction in cell yield (9.8 ± 1 g fr. wt. per flask; control 10.2 ± 1.1 g fr. wt. per flask). 180 g of such mung bean cells were extracted, the extract clarified with charcoal and **1** isolated by PC (solvents L_2, L_3, L_1, L_1, L_4), silica gel TLC (L_5, L_6) and cellulose TLC (L_7, L_8). **1** was characterized by cochromatography, paper electrophoresis and UV spectra before and after $NaBH_4$ reduction. The yield amounted to *ca* 0.03 μmol/g fr. wt which can only be explained by endogenous synthesis.

Differences in nicotinic acid degradation

The data on $^{14}CO_2$-formation from [carboxyl-^{14}C]-nicotinic acid by the cell cultures listed in Fig. 2 allowed the distinction of two groups of cultures. All 29 cell cultures from which trigonelline had been isolated (Fig. 2) produced no $^{14}CO_2$ or only insignificant amounts of less than 0.1% [13]. On the other hand, practically all of the 21 arabinoside producing cell cultures showed considerable $^{14}CO_2$ formation. While some cultures showed medium values between 0.2 and 0.5%, rates went up as high as 22% (Table 1). Since production of $^{14}CO_2$ from carboxyl-labelled nicotinic acid must not necessarily indicate degradation of the pyridine ring structure as well [17, 18], various parallel experiments with [6-^{14}C]-nicotinic acid have also been performed (Table 1). Generation of $^{14}CO_2$ from the ring turned out to be 30–50% lower than from the carboxyl group indicating that decarboxylation might be an early step in nicotinate degradation. The data prove the general capability for nicotinate degradation by those cell suspension cultures which produce the arabinoside.

Experiments with 6-hydroxynicotinic acid

In previous experiments, parsley cell suspension cultures have failed to oxidize [6-^{14}C]-6-hydroxynicotinic acid [4], though this acid has been demonstrated in microbial systems as an important intermediate in nicotinate degradation [19]. Further studies using the nicotinate-degrading cell suspension cultures of *Petroselinum crispum*, *Galium verum*, *Nicotiana tabacum* and *N. glauca* (see Table 1) were carried out with [6-^{14}C]-6-hydroxynicotinic acid (10^{-5} M; 0.5 μCi). Though uptake of the substrate by the cells was nearly quantitative, the 6-hydroxynicotinic acid could be reisolated as the sole compound. $^{14}CO_2$-values obtained with *P. crispum*, *N. tabacum* and *N. glauca* cell cultures were only 0.6, 0.2 and 0.15%, respectively. Considering the very substantial nicotinic acid degradation by *Galium verum* cell cultures (Table 1), the 0.5% of $^{14}CO_2$ obtained with [6-^{14}C]-6-hydroxynicotinic acid indicates that this acid is not a free intermediate in nicotinate catabolism in this and the other cultures.

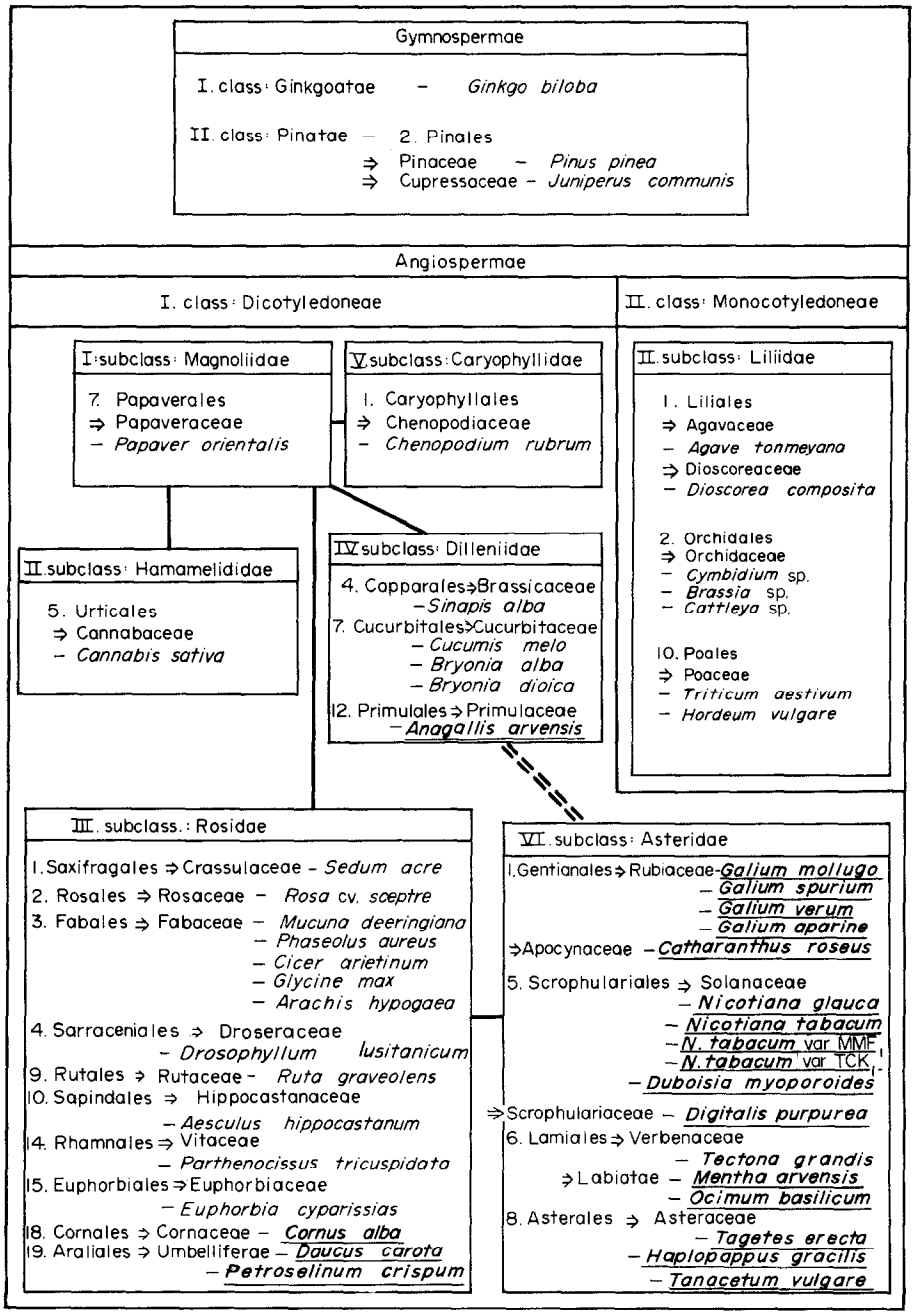


Figure 2. Scheme for distribution of trigonelline—(plant names italics) and nicotinic acid-arabinoside—(plant names bold italics and underlined) formation in plant cell suspension cultures after application of nicotinic acid.

Feeding experiments with trigonelline and nicotinic acid arabinoside

The mutually exclusive formation of **1** and **2** has led to cross feeding experiments with [carboxyl-^{14}C]-nicotinic acid-*N*-arabinoside to trigonelline-producing cell cultures and with both [carboxyl-^{14}C]-trigonelline and [methyl-^{14}C]-trigonelline to cell cultures forming **2**. It was anticipated that the cell suspension cultures would preferentially metabolize their species-characteristic substrate, though the oxidation of a variety of unnatural substrates in cell cultures has been demonstrated [26]. When cell suspension cultures of *Glycine max*, *C. arietinum* and *Ph. aureus* were given [carboxyl-^{14}C]-nicotinic acid-*N*-arabinoside (10^{-3} M, 0.2 μCi) for 60 hr, good uptake of substrate but no $^{14}CO_2$ could be measured. Careful PC analyses (L_1, L_2) revealed no other labelled compounds than **2**; trigonelline was especially shown to be absent. Equivalent experiments with [carboxyl-^{14}C]-trigonelline (10^{-5} M, 2 μCi), however, indicated a certain degree of metabolism of **1**. In cell suspension cultures of *Nicotiana glauca*, radioactivity was found in CO_2 (0.1 %), pyridine nucleotides (1.5 %), nicotinic acid/

Table 1. Degradation of carboxyl- and ring-labelled nicotinic acid by plant cell suspension cultures during 48 hr

Cell culture	$^{14}CO_2$ (%) from decarboxylation of nicotinic acid	ring cleavage
Petroselinum crispum	6.3	4.6
Daucus carota	0.6	0.43
Nicotiana tabacum	0.35	0.2
N. tabacum MMF_1	1.3	n.d.
N. glauca	0.7	0.57
N. tabacum TCK_1	0.7	n.d.
Galium verum	22	14.4
Haplopappus gracilis	2.1	0.15
Catharanthus roseus	2.5	n.d.
Digitalis purpurea	0.9	n.d.
Tanacetum vulgare	1.2	n.d.
Tectona grandis	2.9	n.d.

Values are averages from at least 3 experiments; n.d. = not determined. The individual cultures contained between 7 and 10 g (fr.wt) of cells.

nicotinamide (0.8%) and trigonelline (97%). In cell suspension cultures of *Galium verum*, radioactivity was determined in CO_2 (10.4%), nicotinic acid *N*-arabinoside (5%), nicotinamide (2%) and reisolated trigonelline (85%).

The actual extent of demethylation of **1** was then measured by using [methyl-^{14}C]-trigonelline (10^{-5} M, 1 μCi) and found to be rather high (*N. glauca* 7 and *G. verum* 11%, respectively). Application of the latter substrate to cell cultures of *G. max*, *Ph. aureus* and *C. arietinum* again led to values (0.4–3%) essentially as previously [3] determined.

Measurement of arabinosyl-transferase

Formation of nicotinic acid from nicotinic acid *N*-arabinoside for transfer into either the pyridine nucleotide cycle or catabolic pathways can be visualized to occur by the recently described UDP-dependent arabinosyl-transferase (yielding UDP-arabinose and nicotinate) isolated from parsley cell suspension cultures [2]. The above data on the mutually exclusive occurrence and partially exclusive metabolism of **1** and **2** forced us to measure this arabinosyl-transferase in several cell suspension cultures (Table 2). The experiments were designed to correlate enzyme activity with the degree of nicotinate degradation by a particular cell culture and with the capability to metabolize either **1** or **2**. The data show that in some cell cultures (*G. verum*, *P. crispum*, *N. tabacum*) efficient production and metabolism of **2** seems to be partially correlated with activity of the nicotinic acid *N*-arabinoside: UDP-arabinosyltransferase. Furthermore, cell cultures which lack the ability to form and to metabolize **2** (*Ph. aureus*, *G. max*, *Ch. rubrum*) show extremely low and insignificant levels of the arabinosyltransferase. On the other hand, the quantitative relation of the arabinosyltransferase between the cell cultures of *P. crispum*, *G. verum*, *N. tabacum*, *D. carota* and *G. max* (Table 2) does not reflect the observed differences in cellular capacity for nicotinate degradation.

DISCUSSION

The systematic analyses of 50 cell suspension cultures from both the Gymnospermae and Angiospermae have demonstrated that the formation of trigonelline and nicotinic acid arabinoside from nicotinic acid is mutually exclusive. Despite the small number of cultures investigated, the data in Fig. 2 indicate that nicotinic acid metabolism could be of chemosystematic value, as a marker at the level of order or subclass. Formation of **2** in the orders Rosidae and Dilleniidae point to the accepted phylogenetic relationship between these orders and the Asteridae [14]. According to Fig. 2, there is a point in the Rosidae and Dilleniidae where the metabolic shift from **1** to **2** occurs.

The chemosystematic difference in nicotinate metabolism is further supported by the observation that degradation of nicotinic acid seems to be restricted to that group of cell suspension cultures which produce the arabinoside (Table 1). In general, $^{14}CO_2$-values from exogenous substrates in different cell cultures are subject to considerable variation due to experimental and physiological differences [22, 23] and should, therefore, be compared with caution. It is, however, highly significant that none of the 21 trigonelline-producing cell suspension cultures formed any notable amount of $^{14}CO_2$ from labelled nicotinate. In contrast, degradation of nicotinate in the other group of cultures could readily be measured (Table 1). Furthermore, **1** or **2** are not metabolically exchangeable because our cross feeding experiments have shown that **2** is no substitute for **1** in the three legume cell cultures investigated. The same

Table 2. Values for nicotinic acid *N*-α-L-arabinoside: UDP-arabinosyltransferase [2] in plant cell suspension cultures and capability of cell cultures to degrade and metabolize nicotinic acid

Cell culture	Protein (mg)	Total activity (nicotinate formed in UDP depending reaction) (nmol/hr)	Specific activity (nicotinate formed) (nmol/mg protein × hr)	Degree of nicotinate degradation (Table 1)	Formation of nicotinic acid-*N*-arabinoside (Fig. 2)
	I	II	III	IV	V
Petroselinum crispum	4.2	21.45	5.1	+ +	+
Galium verum	5.9	2.44	0.41	+ +	+
Nicotiana tabacum	0.9	0.31	0.34	+	+
Daucus carota	2.8	0.1	0.04	+	+
Glycine max	6.1	0.82	0.13	—	—
Phaseolus aureus	6.2	0.29	0.04	—	—
Chenopodium rubrum	5.6	0.17	0.03	—	—

Formation of nicotinic acid from **2** was measured and suitable controls without UDP were also run.

has previously been found for **1** in parsley cell cultures [4]. Metabolism of [carboxyl-^{14}C]- and [methyl-^{14}C]-trigonelline in *G. verum* and *N. glauca* is rather pronounced and leads to free nicotinic acid. According to expectation, this pool of free nicotinate is partly degraded and partly converted into **2**. One must however, question the specificity of the observed *N*-demethylation because cell suspension cultures, especially *N. gauca* have repeatedly been shown to *N*-demethylate a wide range of substrates [26]. Enzymatic evidence with plant mixed function oxidases have resulted in the observation that various exogenous substrates will be *N*-demethylated [31, 32]. It appears that removal of the *N*-methyl group is more facile than splitting of the *N*-arabinose, which is corroborated by the absence of the arabinosyl-transferase from trigonelline-producing cell cultures (Table 2). Further enzymatic experiments are needed to fully explain these discrepancies.

In comparison to the high values obtained with labelled nicotinic acid in cell suspension cultures of *P. crispum* and *G. verum* (Table 1), the very low yield of $^{14}CO_2$ found with [6-^{14}C]-6-hydroxynicotinic acid clearly excludes this latter compound as an intermediate catabolite. One may, therefore, visualize a catabolic sequence of nicotinic acid in plant cells where (oxidative) decarboxylation precedes both substitution adjacent to the nitrogen atom and ring cleavage reactions. This assumption is supported by the constantly much higher rate of CO_2 from the carboxyl group than from the ring position (Table 1) and, furthermore, by the natural occurrence of compounds such as 3-methoxypyridine [24]. Enzymatic evidence for nicotinate decarboxylation has been obtained [17] and must be substantiated for cell cultures; attempts to isolate pyridine derivatives from nicotinate degrading cell suspension cultures have so far been unsuccessful [25 and unpublished]. The present experiments do not, however, exclude the intermediate occurrence of glycosylated forms of 6-hydroxynicotinic acid. Though the nicotinic acid *N*-α-L-arabinoside: UDP-arabinosyltransferase [2] seems to be present only in nicotinic acid degrading cell suspension cultures (Table 2), removal of arabinose from **2** must not necessarily precede nicotinate catabolism. A quantitative comparison between rate of nicotinic acid degradation (Tables 1 and 2) and level of enzyme (Table 2) might indicate that **2** and not free nicotinic acid is introduced into catabolic pathways. Due to the strict compartmentation of plant cells [26] or an expected rigid specificity of arabinoside-synthesizing enzymes, such an assumption could well explain the poor degradation of exogenously applied 6-hydroxynicotinic acid.

The restricted occurrence and seeming chemosystematic importance of **1** and **2** in cell suspension cultures (Fig. 2) are in a certain contrast to the extremely wide distribution of **1** in the plant kingdom [8, 9]. **1** has, therefore, been denied any systematic value [8]. Though some reports on trigonelline isolation can be questioned [13] due to outdated analytical techniques, the repeated isolation of **1** [8, 9] from *Coffea arabica* (Rubiaceae), *Solanum tuberosum* (Solanaceae), *Stachys silvatica* (Labiatae) or *Dahlia variabilis* (Compositae) cannot be reconciled with the distribution given in Fig. 2. This contrast could, however, originate from differences between cell suspension cultures and intact plants. Various examples for differences in metabolism and in occurrence of compounds between cell cultures and intact plants can be cited [27, 28]. In most cases, reasons for these differences are unknown. In plants, trigonelline is mostly found in storage organs (roots, seeds, old leaves) where a permanent physiological role such as found in cell suspension cultures can perhaps be excluded. Thus, application of [U-methyl-^{14}C]-trigonelline to trigonelline-containing leaves of *Coffea arabica* did not result in any metabolism of **1** [29]. On the other hand, formation and occurrence of **2** in intact plants has still to be shown. Preliminary data with tobacco seedlings [13] indicate that the arabinoside might be present. In this connection it will also be essential to determine in the tobacco plant the metabolic relationship between **2** and the nicotinic acid-*N*-glucoside described by Mizusaki *et al.* [30]; the latter compound could not be found in our four lines of *Nicotiana* cell cultures.

EXPERIMENTAL

Reagents. [Carboxyl-^{14}C]-nicotinic acid (spec. act. 59 mCi/mmol) and [6-^{14}C]-nicotinic acid (spec. act. 45 mCi/mmol) were obtained from Radiochemical Centre, Amersham. [Carboxyl-^{14}C]-nicotinic acid-*N*-α-L-arabinoside (spec. act. 4.5 mCi/mmol), [6-^{14}C]-6-hydroxynicotinic acid (spec. act. 0.6 mCi/mmol), [methyl-^{14}C]-trigonelline (spec. act. 0.3 mCi/mmol) and [carboxyl-^{14}C]-trigonelline (spec. act. 2.48 mCi/mmol) have previously been synthesized by cell culture or preparative methods [2–4]. All other chemicals were of commercial origin.

Cell cultures. Growth and cultivation of the laboratory's collection of cell suspension cultures have previously been described [2–4, 22, 23]. Whenever necessary nicotinic acid was omitted from the media. Cell cultures were used in the late logarithmic growth phase. Cell suspension cultures from other laboratories were used in their growth medium [13] and prior to the experiments they were shaken on a gyrotory shaker (120 rpm, 27°) for 24 hr in darkness. The application of substrates (autoclaved or filter sterilized) under aseptic conditions, collection and measurement of $^{14}CO_2$ as well as the determination of sterility of the cell cultures followed previous reports [22, 23].

Isolation of metabolites. Cells were obtained by filtration, 3 × washed with H_2O and homogenized with an ultraturrax in hot 80% EtOH for 1 min. The insoluble cell material were 3 times further extracted with 80% EtOH under reflux. The combined filtrates were reduced under vacuum and used for chromatographic separation. Pyridine nucleotides were extracted in the cold with $HClO_4$. Identification of compounds investigated has been published [2–4].

Chromatographic solvents. PC was carried out on EDTA prewashed Whatman 3 MM paper with the solvent systems L_1: 60% *n*-PrOH and L_2: 85% *i*-PrOH. Continuous PC with system L_2 for 80 hr well separates **1** and **2**. L_3: *n*-BuOH sat. with 25% NH_3; L_4: *n*-BuOH–HOAc–H_2O, 4:1:5. Solvents for prep. TLC on Si gel were L_5: H_2O–EtOH–MeCOEt, 3:3:1; L_6: EtOAc–MeCOEt–HCOOH, 7:2:1. Solvents for TLC on cellulose were: L_7: MeOH–HOAc, 9:1; L_8: C_6H_6–HOAc, 45:25:4. Labelled compounds on paper chromatograms were located according to published methods [2].

Paper electrophoresis. According to previous reports [2].

Radioactivity. $^{14}CO_2$, soluble and insoluble samples were measured for radioactivity as previously described [2, 22].

Enzymatic studies. The nicotinic acid-*N*-α-L-arabinoside: UDP-arabinosyltransferase was essentially measured as previously described [2] using protein preparation after precipitation with 80% ammonium sulfate in potassium phosphate buffer (0.1 M; pH 7.0).

Acknowledgements—Our sincere thanks for generous help with various plant cell suspension cultures are due to Profs. Becker and Bopp, Heidelberg, Friedrich, Schulte and Leistner, Münster, Bergmann, Cologne, Reinhard, Tübingen, Zenk, Bochum

Neumann, Giessen and Dr. Hörster, Münster. Financial support by Deutsche Forschungsgemeinschaft and Fonds der Chemischen Industrie is gratefully acknowledged.

REFERENCES

1. Leienbach, K.-W., Heeger, V., Neuhann, H. and Barz, W. (1975) *Plant. Med.*(Suppl.), 148.
2. Leienbach, K.-W. and Barz, W. (1976) *Hoppe-Seyler's Z. Physiol. Chem.* **357**, 1069.
3. Heeger, V., Leienbach, K.-W. and Barz, W. (1976) *Hoppe-Seyler's Z. Physiol. Chem.* **357**, 1081.
4. Leienbach, K.-W., Heeger, V. and Barz, W. (1976) *Hoppe-Seyler's Z. Physiol. Chem.* **357**, 1089.
5. Neuhann, H., Leienbach, K.-W. and Barz, W. (1979) *Phytochemistry*, **18**, 61.
6. Schütte, H. R. (1969) in *Biosynthese der Alkaloide* (Mothes, K. and Schütt, H. R., eds.) pp. 137–138. VEB Deutscher Verlag der Wissenschaften, Berlin.
7. Karrer, P. (1958) in *Konstitution und Vorkommen der organischen Pflanzenstoffe*, 2nd edn. Birkhäuser, Basel-Stuttgart, p. 997.
8. Hegnauer, R. (1963) in *Chemotaxonomie der Pflanzen*, Vol. 2. Birkhäuser, Basel.
9. Gibbs, R. D. (1974) in *Chemotaxonomy of Flowering Plants*, Vol. 1, p. 290. McGill-Queen's Univ. Press, Montreal.
10. Antony, A., Gopinathan, K. P. and Vaidyanathan, C. S. (1975) *Indian J. Exp. Biol.* **13**, 39.
11. Beers, J. R. (1967) *Comp. Biochem. Physiol.* **21**, 11.
12. Gupta, K. C. and Miller, R. L. (1977) *Lloydia* **40**, 303.
13. Willeke, U. (1976) Staatsexamensarbeit, Universität Münster.
14. Ehrendorfer, F. (1971) in *Lehrbuch der Botanik* (Denffer, D. v., Schumacher, W., Mägdefrau, K. and Ehrendorfer, F., eds.) 30th edn, pp. 666–745. G. Fischer, Stuttgart.
15. Hüsemann, W. and Barz, W. (1977) *Physiol. Plant.* **40**, 77.
16. Boll, H. J. (1978) Staatsexamensarbeit, Universität Münster.
17. Chandler, J. L. R. and Gholson, R. K. (1972) *Phytochemistry* **11**, 239.
18. Gross, D. (1970) *Fortschr. Chem. Org. Naturst.* **28**, 109.
19. Gross, D. (1969) in *Biosynthese der Alkaloide* (Mothes, K. and Schütte, H. R., eds.) Chapter 8. VEB Deutscher Verlag der Wissenschaften, Berlin.
20. Gamborg, O. L., Miller, R. A. and Ojima, K. O. (1968) *Exp. Cell Res.* **50**, 151.
21. Gamborg, O. L. (1966) *Can. J. Biochem.* **44**, 791.
22. Berlin, J. and Barz, W. (1971) *Planta* **98**, 300.
23. Harms, H., Haider, K., Berlin, J., Kiss, P. and Barz, W. (1971) *Planta* **105**, 342.
24. Fugster, C. H., Griot, R. and Karrer, P. (1953) *Helv. Chim. Acta* **36**, 474.
25. Leienbach, K.-W. (1976) Dissertation, Universität Münster.
26. Barz, W. (1977) in *Plant Tissue Culture and its Biotechnological Application* (Barz, W., Reinhard, E. and Zenk, M. H., eds.) pp. 153–171. Springer Berlin.
27. Teuscher, E. (1973) *Pharmazie* **28**, 6.
28. Overton, K. (1977) in *Plant Tissue Culture and its Biotechnological Application* (Barz, W., Reinhard, E. and Zenk, M. H., eds.) Springer, Berlin, pp. 66–75
29. Wanner, H., Keude, H. and Fahrni, P. (1960) *Flora (Jena)* **149**, 162.
30. Mizusaki, S., Tanabe, Y., Kisaki, T. and Tamaki, E. (1970) *Phytochemistry* **9**, 549.
31. Frear, D. S., Swanson, H. R. and Tanaka, F. S. (1972) *Rec. Adv. Phytochem.* **5**, 225.
32. Young, O. and Beevers, H. (1976) *Phytochemistry* **15**, 379.