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Dihydrocaffeoyl Polyamines (Kukoamine and Allies) in Potato (*Solanum tuberosum*) Tubers Detected during Metabolite Profiling

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Four related phenolic amides previously undescribed from the species were revealed during metabolic profiling of potato (*Solanum tuberosum*) tubers. N^1,N^{12}-Bis(dihydrocaffeoyl)spermine (kukoamine A) and N^1,N^8-bis(dihydrocaffeoyl)spermidine were positively identified by comparison with authentic standards, while the structures N^1,N^4,N^{12}-tris(dihydrocaffeoyl)spermine and N^1,N^4,N^8-tris(dihydrocaffeoyl)spermidine are proposed for the other two metabolites. Each amide was present at several tens of micrograms per gram of dry matter. Several of these compounds were subsequently detected in other solanaceous species, such as tomato (*Lycopersicon esculentum*) and *Nicotiana sylvestris*. They appeared not to be present in *Arabidopsis thaliana* or *Beta vulgaris*. Bis(dihydrocaffeoyl)spermine isomers have previously been identified in only a single plant, the Chinese medicinal species *Lycium chinense* (Solanaceae), where they may account for some of the described biological activity. The other compounds have not until now been reported in vivo, though some of the equivalent hydroxycinnamoyl derivatives are known. The surprising discovery of kukoamine and allies in a range of solanaceous species including potato, a common food crop that has a long history of scientific investigation, provides exemplary evidence for the potential of the nontargeted techniques of metabolomics in studying plant metabolites.

KEYWORDS: Kukoamine; metabolite profiling; polyamine conjugates; potato; spermidine; spermine

INTRODUCTION

Secondary metabolites of plant origin have long been of interest to man, one of the major reasons in recent years being their use, either directly as precursors or as lead compounds, in the pharmaceutical industry (*1*). The potential health benefits of certain groups of secondary metabolites naturally present in the diet are now also attracting considerable attention (*2*−*4*). Detailed study of the chemical composition of plants therefore has a long-established tradition. Such studies are now taking on yet further dimensions as they are being used within the disciplines of functional genomics and systems biology to probe gene function and coordination within living organisms in their complex natural environment (*5*−*10*).

Until recently, the thorough phytochemical examination of plants has been a time-consuming process, involving careful extraction and fractionation steps after which it is then possible to identify and characterize either a relatively few major unknowns or to confirm the presence of, and quantitate, a somewhat larger range of metabolites for which pure standards are available. Despite the inherent importance of phytochemicals, very few plant species have thus so far been examined in any great degree of depth, and surprisingly few of these are crop species. The developing interest in functional genomics and systems biology has however seen something of a shift in the modern analytical approach, with techniques being developed that allow the simultaneous, rapid, sensitive, and relatively nontargeted analysis of a wide range of metabolites, perhaps ultimately with a view to being able to understand the full metabolic complement, or metabolome, of a plant species (*5*−*7*). These metabolomic techniques are typically based around NMR, LC/MS, or GC/MS (*5*−*10*), with integrated analytical approaches such as combined LC/MS/NMR now also being developed (*11*−*13*). Such methodologies offer considerable advantages to the field of phytochemistry in that they make few assumptions as to the chemical nature of metabolites that will be detected, and with appropriate data-handling techniques, a very considerable depth of analysis is possible (*14*, *15*). They also greatly facilitate the spread of species that can readily be investigated.

In the present paper, we describe the identification of significant amounts of a potentially bioactive phenolic polyamine conjugate, kukoamine A, and related compounds in wild-type

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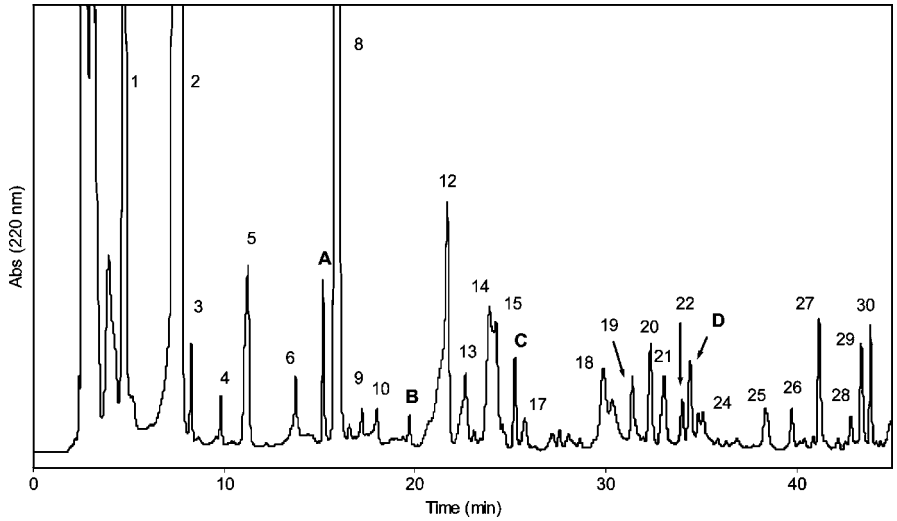

Figure 1. HPLC/DAD profile of a potato extract monitored at 220 nm. Major peaks that have been characterized in addition to A–D are as follows: 2 = tyrosine; 5 = phenylalanine; 6 = *trans*-caffeoylputrescine; 8 = tryptophan; 9 = *trans*-feruloylputrescine; 10 = 3-*O*-caffeoyl quinate; 12 = chlorogenic acid; 13 = 4-*O*-caffeoyl quinate; 14 = caffeic acid; 18 = pelargonidin 3-*O*-(coumaroylrutinose)-5-*O*-glucoside.

potato tubers that were detected during metabolomic studies primarily designed to assess the compositional changes occurring after genetic modifications had been made to different metabolic pathways (*16*). Kukoamines had previously only been described from the medicinal plant *Lycium chinense* (*17, 18*), though they have been attracting attention because of their hypotensive effects (*17*) and an antitrypanosomal action mediated by an inhibition of trypanothione reductase (*19*). The discovery by us that they are distributed far more widely among the Solanaceae and occur in one of the staple food crops of the Western world exemplifies how the nondirected analytical techniques of metabolomics may provide insights into a plant's metabolic complement and the wider significance of this, both at the pure and applied levels.

MATERIALS AND METHODS

Chemicals. Chemically synthesized isomers of kukoamine and their spermidine equivalents were kindly provided by Dr. Dionissios Papaioannou, University of Patras, Greece. Dihydrocaffeic acid was obtained from Apin Chemicals Ltd. (Abingdon, UK) and methyl vanillate from Aldrich (Gillingham, UK).

Biological Materials. Potato (*Solanum tuberosum*) tubers of cv. Desiree used for HPLC/diode array detector (HPLC/DAD) studies were from plants grown in pots and maintained under polytunnels at the Scottish Crop Research Institute (SCRI), Invergowrie, Scotland, during 1999. The more extensive range of samples for LC/MS analyses (comprising commercial cultivars and additional material from the Commonwealth Potato Collection) were from plants grown in the field at SCRI during 2002. Samples of *Nicotiana sylvestris* leaf were from Prof. Michel Jacobs, The Free University of Brussels, and tomato fruits (*Lycopersicon esculentum* cv. Ailsa Craig) were provided by Prof. Peter Bramley, University of London.

Sample Preparation. Potato tubers were prepared, freeze-dried, and powdered according to the methods of Defernez et al. (*16*); samples were then stored at −20 °C. For HPLC/DAD studies, aliquots of powder were extracted according to the method of Defernez et al. (*16*). For LC/MS metabolomic studies, this method was simplified slightly, powdered freeze-dried tubers or other plant material being extracted with 70% aqueous methanol containing 20 mg/L methyl vanillate as internal standard for 30 min at room temperature (typically using 50 mg of plant material/mL). Samples were centrifuged briefly in a benchtop centrifuge to remove debris, then the supernatant was diluted to 40% methanol immediately prior to analysis. The two extraction procedures produced very comparable results.

HPLC Profiling and Quantitation of Metabolites. Typically 20−50 μL of extract was used for analysis. HPLC was carried out on a Spectra-Physics HPLC system using a 250 × 4.6 mm i.d. Columbus C18 column (Phenomenex, Macclesfield, UK). This was equilibrated with 1 mM aqueous trifluoroacetic acid (solvent A) and eluted with a gradient of increasing acetonitrile (solvent B) at a flow of 1.0 mL/min. Gradient conditions were as follows: time 0, 100% A; 40 min, 68% A, 32% B; 45 min, 50% A, 50% B; 50 min, 100% A. The eluant was monitored with a diode array detector over the range 200−360 nm. Kukoamines and analogues were quantitated by peak area at 220 nm.

LC/MS and LC/MS/MS. LC/MS was conducted using a Micromass Quattro II triple quadrupole mass spectrometer (Waters, Manchester, UK) coupled to a Jasco PU-1585 HPLC equipped with an AS-1559 cooled autoinjector. The column employed was a 150 × 4.6 mm i.d., 3 μm, Luna C18(2) column (Phenomenex). This was equilibrated with 1 mM aqueous trifluoroacetic acid (solvent A) and eluted with a gradient of increasing acetonitrile (solvent B) at a flow of 0.7 mL/min. Gradient conditions were as follows: time 0, 100% A; 40 min, 68% A, 32% B; 45 min, 100% B; 50 min, 100% B; 52 min, 100% A. The column effluent was passed though an ASI 620 fixed-ratio splitter valve (Presearch, Hitchin, UK), and the majority was sent to a UV detector with the remaining 120 μL/min entering the mass spectrometer. Spectra were obtained in positive ion electrospray mode using a Micromass Z-spray ion source. The electrospray probe was operated at 3.5 kV and a cone voltage of 28 V. The source and desolvation temperatures were 120 and 350 °C, respectively. The nitrogen nebulizing and drying gas flow rate were optimized at 15 and 500 L/h, respectively. Spectra were recorded (in centroid mode) between *m*/*z* 50 and 1500 with a scan duration of 2 s/scan and an interscan time of 0.1 s. MS1 was set to unit mass resolution or better (LM and HM resolution parameters both set to 15.0).

Conditions for LC/MS/MS were the same as for LC/MS with the following differences: argon gas was introduced into the collision cell of the mass spectrometer at a pressure of 0.08 Pa. Product ion spectra of *m*/*z* 474 were recorded at a collision energy of 35 eV in the retention time window 17.0−23.0 min. Spectra were acquired in raw data acquisition mode between *m*/*z* 50 and 500 at a rate of 1 scan/s and an interscan time of 0.1 s. Spectra were averaged and smoothed using MassLynx 3.4 software (Waters, Manchester, UK).

RESULTS AND DISCUSSION

Identification of Kukoamine and Allies. Reverse phase HPLC/DAD profiling of aqueous methanolic extracts of potato (cv. Desiree) tubers revealed approximately 30 major UV-

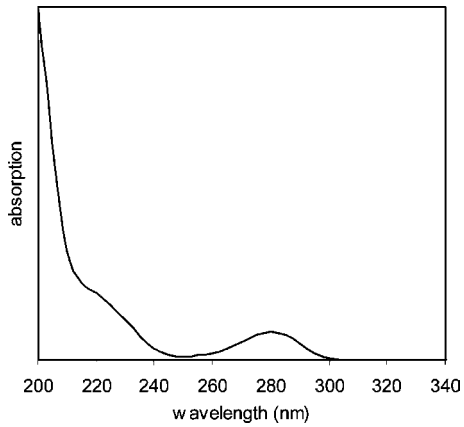

Figure 2. UV spectrum of unknowns A–D.

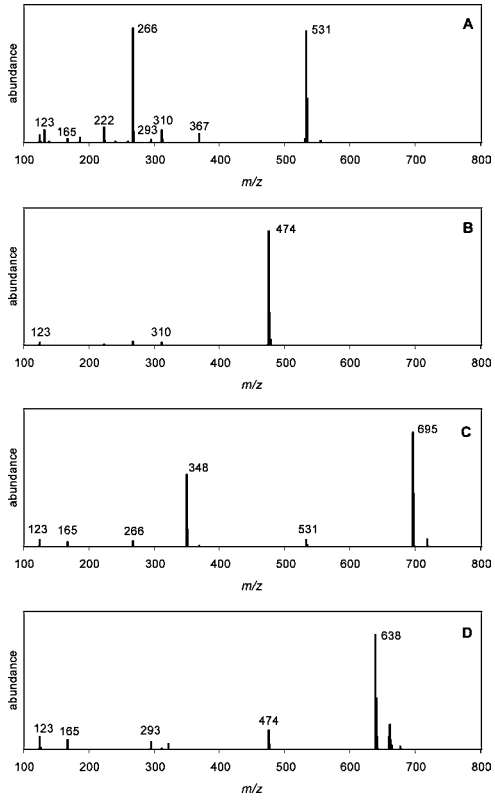

Figure 3. Mass spectra of the four unknowns A–D from potato.

absorbing peaks and several more minor components (**Figure 1**). Equivalent LC/MS profiles revealed some 200 peaks after appropriate background subtraction and peak deconvolution, indicative of the anticipated greater sensitivity and broader selectivity. For instance, due to their low UV absorption, glycoalkaloids were only adequately seen during LC/MS. The identity of many of the major peaks (*16*) could be determined readily from the mass spectra and by reference to commercially available standards and previous literature on potato metabolites (*20*−*24*). However, a number of important peaks, both in terms of UV absorption and MS ion intensity, were initially unassigned. Notable among these were four peaks, labeled A−D in **Figure 1**, which shared a similar UV spectrum (**Figure 2**) suggestive of an *o*-dihydroxyphenol (*25*). The mass spectra of these compounds contained prominent high mass ions at *m*/*z* 531, 474, 695, and 638, respectively (**Figure 3**), that appeared to represent the molecular ions MH^+ (minor ions at higher mass could be attributed to Na^+ adducts). Peaks corresponding to the molecular ions of compounds A and B also appeared as fragment ions in the spectra of compounds C and D, respectively (**Figure 3**). This information indicated that the four compounds possessed molecular weights of 530, 473, 694, and 637 and apparently fell into two subclasses, with the two higher molecular weight forms probably being derivatives of the lower molecular weight forms. Since our metabolomic studies had indicated that the levels of these compounds were important factors in helping to distinguish between certain potato lines (*16*), there was some incentive to fully establish their chemical identity.

The odd-numbered molecular weights of compounds B and D indicated that these must contain an odd number of nitrogen atoms (*26*). Compounds A and C, which yielded abundant doubly charged protonated ions $[MH_2]^{2+}$ (at *m*/*z* 266 and 348, respectively) also appeared to be nitrogenous, but they contained an even number of nitrogen atoms (abundant doubly charged molecules in electrospray spectra often indicate the presence of multiple basic sites). Although the ESI LC/MS spectrum of compound B was dominated by the MH^+ ion at *m*/*z* 474, LC/MS/MS yielded a number of fragments that also appeared in the ESI LC/MS spectrum of compound A, notably at *m*/*z* 222, 165, and 123 (**Tables 1** and **2**). It was therefore highly probable that compounds A−D were chemically related and that compound B was the parent compound, to which further groups were then added. The MW difference between compounds A and B was 57, suggestive of an aminopropyl group and raising the possibility that spermidine and spermine might be involved, these being common amines differing by an aminopropyl residue. The difference between compounds A and C and compounds B and D was 164. Because of the UV evidence for the presence of an *o*-dihydroxyphenol moiety, it was tentatively proposed that this fragment might relate to a dihydroxyphenylpropyl group. Supporting evidence for this hypothesis was provided by the loss of a fragment of mass 164 in the mass spectrum of compound A. Combining all the spectroscopic evidence thus led us to conclude that the series of compounds were probably related to bis(dihydrocaffeoyl)spermine (compound A), bis(dihydrocaffeoyl)spermidine (compound B), tris(dihydrocaffeoyl)spermine (compound C), and tris(dihydrocaffeoyl)spermidine (compound D). Isomers of *N*,*N*′-bis(dihydrocaffeoyl)spermines, referred to as kukoamines A and B, have previously been isolated from *L. chinense* (Solanaceae) (*17*, *18*) and shown to possess a range of biological activities (*17*, *19*).

Because of the medicinal interest in dihydrocaffeoyl polyamines, the various structural isomers of the bis(dihydrocaffeoyl) derivatives have now been synthesized (*19*, *27*, *28*), and we were able to use these chemical standards to test the proposed structures of the potato unknowns. The equivalence of UV and MS spectroscopic properties and HPLC retention times with certain of these standards confirmed that compounds A and B, and by implication also C and D, were indeed dihydrocaffeoyl polyamines. Compound A was identified on the basis of its HPLC retention time and MS fragmentation pattern (**Table 1**) as N^1,N^{12}-bis(dihydrocaffeoyl)spermine (**Figure 4**), otherwise known as kukoamine A. Compound B could not be identified directly from its basic mass spectrum as the various isomers of *N*,*N*′-bis(dihydrocaffeoyl)spermidine showed similar behavior. However, small but reproducible differences in MS/MS fragmentation patterns and in HPLC retention time (**Table 2**) revealed compound B to be N^1,N^8-bis(dihydrocaffeoyl)spermidine (**Figure 4**). Although no authentic standards were available for confirmation, on the basis of the known structure of compounds A and B as well as previous precedents for this

Table 1. HPLC Retention Times and LC/MS Spectra Recorded for Bis(dihydrocaffeoyl)spermine Standards and for the Related Metabolite Present in Potato

compound	t_R, min	mass spectra, *m/z* (%)
N^1,N^4 isomer	14.7	531 (100), 529 (3), 367 (33), 310 (3), 266 (80), 257.5 (2), 222 (5), 184 (12), 165 (9), 123 (23)
N^1,N^9 isomer	14.3	531 (99), 529 (2), 367 (4), 310 (6), 266 (100), 257.5 (91), 249 (1); 222 (12), 184 (2), 165 (2), 123 (6)
N^1,N^{12} isomer	14.4	531 (72), 529 (2), 367 (7), 310 (11), 266 (100), 257.5 (1), 222 (12), 184 (5), 165 (2), 123 (6)
N^4,N^9 isomer	15.4	531 (62), 529 (13), 367 (5), 310 (2), 266 (77), 257.5 (100), 249 (51), 222 (4), 184 (1), 165 (1), 123 (5)
potato metabolite A	14.4	531 (97), 529 (4), 367 (8), 310 (10), 266 (100), 257.5 (1), 222 (14), 184 (4), 165 (2), 123 (7)

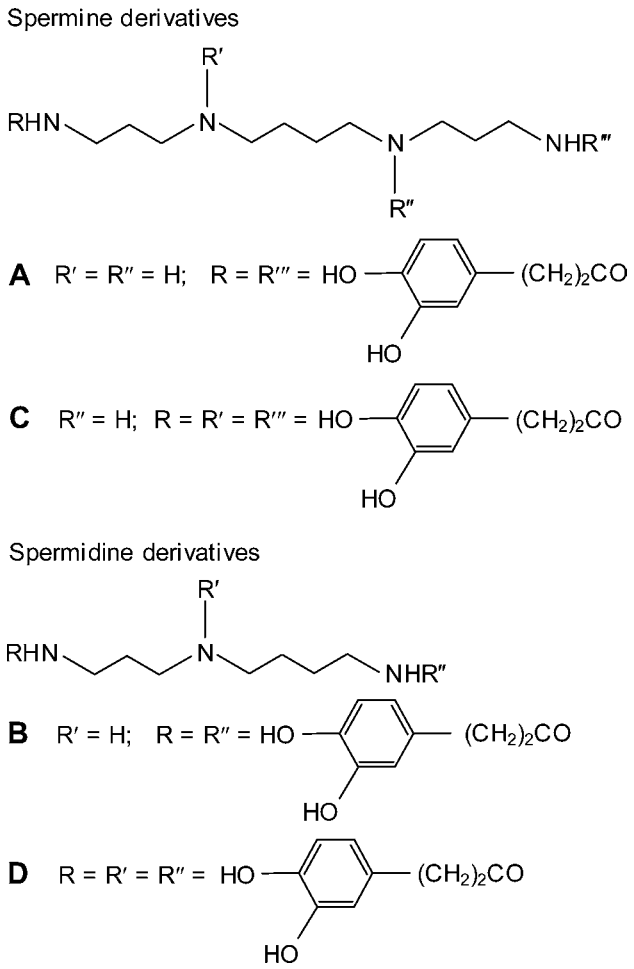

Figure 4. Proposed structures of dihydrocaffeoyl conjugates A–D.

general class of metabolite (*29*), compounds C and D were thought to represent *N*,*N*′,*N*″-tris(dihydrocaffeoyl) derivatives. The mass spectral properties of compound C suggested that it was probably N^1,N^4,N^{12}-tris(dihydrocaffeoyl)spermine (**Figure 4**). This deduction is supported by the lack of the strong ion due to $[MH_2 - NH_3]^{2+}$ that is seen in the mass spectrum of bis(dihydrocaffeoyl)spermines that carry a N^9-dihydrocaffeoyl group and a free N^{12} primary amine (**Table 1**). Compound D could be assigned the structure N^1,N^4,N^8-tris(dihydrocaffeoyl)-spermidine, as only one relevant isomer exists (**Figure 4**).

Table 2. HPLC Retention Times and LC/MS/MS[a] Spectra Recorded for Bis(dihydrocaffeoyl) Spermidine Standards and for the Related Metabolite Present in Potato

compound	t_R, min	mass spectra, *m/z* (%)
N^1,N^4 isomer	18.8	457 (2), 293 (4), 236 (4), 222 (100), 165 (24), 123 (16), 112 (6), 100 (4), 72 (42)
N^1,N^8 isomer	18.9	457 (1), 293 (1), 236 (6), 222 (100), 165 (41), 123 (24), 112 (3), 100 (5), 84 (1), 72 (7)
N^4,N^8 isomer	19.8	457 (3), 293 (1), 265 (4), 236 (12), 222 (100), 165 (45), 123 (29), 112 (5), 100 (5), 84 (3), 72 (8)
potato metabolite B	18.9	457 (2), 293 (1), 236 (6), 222 (100), 165 (42), 123 (25), 112 (4), 100 (7), 84 (1), 72 (7)

[a] Monitoring daughters of *m/z* 474; all isomers of *N*,*N*′-bis(dihydrocaffeoyl) spermidines give LC/MS spectra strongly dominated by the molecular ion $MH^+ = 474$.

The amounts of chemically synthesized kukoamines and analogues at our disposal were too small to allow reliable quantitation of dihydrocaffeoyl polyamines in potato tuber extracts using these as standards. Since the dihydroxyphenyl chromophore is unlikely to be greatly affected by formation of amides (the phenolic ring and the carboxylic acid group not being in conjugation in dihydrocaffeic acid, unlike the situation with caffeic acid), then free dihydrocaffeic acid could be used for quantitation on the basis of UV absorption. Levels of individual dihydrocaffeoyl polyamines in tubers of cv. Desiree were typically found to be several tens of micrograms of dihydrocaffeic acid equivalents per gram of dry weight (DW). After adjustment to allow for the polyamine part of the molecules, the average levels for 28 samples were compound A = 57 μg/g DW, B = 26 μg/g DW, C = 32 μg/g DW, and D = 58 μg/g DW. These values compare with typical total glycoalkaloid contents (solanine + chaconine) of 50−500 μg/g DW in many potato cultivars (*30*) assuming dry matter content to be roughly 20% of fresh weight.

Varietal Differences in Dihydrocaffeoyl Polyamine Content. Tubers of some 20 varieties of potato were examined for their relative dihydrocaffeoyl polyamine content using LC/MS (**Table 3**). Most varieties contained levels of individual metabolites that were broadly similar to those found in Desiree, though there were a few exceptions. The variety Cara, for instance, was characterized by a low level of compound A (N^1,N^{12}-bis(dihydrocaffeoyl)spermine). Brodick and Shelagh possessed low level of compound C (tris(dihydrocaffeoyl)-spermine), while Pentland Dell, Stirling, and the two diploid *Solanum phureja*-derived varieties Inca Sun and Mayan Gold had low levels of compound D (tris(dihydrocaffeoyl)spermidine). Golden Wonder possessed both a significantly elevated level of compound C and a low level of compound D.

Other Dihydrocaffeoyl Polyamines. *In Potato*. The series of dihydrocaffeoyl polyamines we identified in potato led us to hypothesise that N^1,N^4,N^9,N^{12}-tetrakis(dihydrocaffeoyl)spermine might also occur. A candidate peak with the correct LC/MS properties ($MH^+ = 859$, strong $[MH_2]^{2+}$, retention time of ca. 39 min) was indeed observed in extracts from several potato varieties but in such small amounts that further characterization was impossible. A putative *N*,*N*′,*N*″-tris(dihydrocaffeoyl)spermidine glycoside ($MH^+ = 800$ with major loss of *m/z* 162, retention time ca. 3 min less than for the aglycon) was also noted in some tubers from one old Chilean line of *S. t. tuberosum* (Commonwealth Potato Collection TBR.3369) whose origin predates modern potato breeding programs. Trace amounts were seen in many other varieties.

Table 3. Relative Dihydrocaffeoyl Polyamine Content of a Range of Potato Varieties[a]

	relative content of metabolite normalized to content in Desiree (=1.0)			
variety	compd A ($MH^+ = 531$)	compd B ($MH^+ = 474$)	compd C ($MH^+ = 695$)	compd D ($MH^+ = 638$)
Anya	0.5	1.1	3.1	1.4
Barbara	0.3	0.6	0.6	0.3
Brodick	0.8	1.7	0.1	1.0
Cara	0.08	1.1	1.7	0.7
TBR.3302	0.7	1.3	0.4	1.1
TBR.3369[b]	0.2	2.4	0.3	1.3
Desiree	1.0	1.0	1.0	1.0
Eden	0.6	1.7	1.3	0.5
Glenna	0.3	0.7	0.6	0.3
Golden Wonder	0.2	0.9	4.9	0.03
Inca Sun[c]	0.4	0.9	0.6	0.03
Lumpers	0.9	1.4	0.7	0.7
Maris Piper	0.2	1.0	2.1	0.2
Mayan Gold[c]	0.3	0.6	0.9	0.05
Morag	0.5	1.3	0.6	0.5
Pentland Crown	0.5	0.7	0.4	0.8
Pentland Dell	0.4	0.5	2.4	0.01
Pentland Javelin	0.4	0.8	1.2	0.8
Pink Fir Apple	0.3	0.7	2.3	0.3
Record	0.4	1.4	0.4	1.2
Shelagh	0.3	1.2	0.07	2.0
Sterling	0.4	1.0	1.0	0.05
Torridon	0.4	0.8	0.5	0.1

[a] Results are expressed as the content of each metabolite relative to that measured in cv. Desiree (see the text for typical values in this cultivar) and are based on the intensities of the molecular ions seen during LC/MS. Figures represent the mean of at least four independent analyses, each involving material from four to six tubers. [b] Average of four clones, some heterogeneity present. [c] Cultivar of *S. phureja.*

In Other Solanaceous Species. The observation that kukoamine A is not restricted simply to *L. chinense*, as once believed, but is also present in another member of the Solanaceae raised the possibility that dihydrocaffeoyl polyamines might be widespread in the family. LC/MS metabolic screening of *N. sylvestris* leaf extracts revealed the presence of small amounts of a bis(dihydrocaffeoyl)spermine, probably kukoamine A, and more substantial amounts of a bis(dihydrocaffeoyl) spermidine (data not shown). In addition, a peak was observed with a UV spectrum indicative of the presence of a dihydroxycinnamoyl group (*25*), and a mass spectrum dominated by the MH^+ ion at *m*/*z* 472. When subject to LC/MS/MS, this gave a spectrum almost identical to that of a *N*,*N*′-bis(dihydrocaffeoyl)spermidine, but with the presence of an additional fragment ion at *m*/*z* 163. This compound thus seemingly represents an *N*-caffeoyl-*N*′-dihydrocaffeoylspermidine, and it may be related to metabolites recently discovered in eggplant (*Solanum melongena*) (*31*). There was little sign of any tris(dihydrocaffeoyl)-substituted metabolites such as those found in potato, though trace amounts of what might be a tricaffeoylspermidine ($MH^+ = 632$) were detected.

In tomato (*L. esculentum*), metabolite profiling of fruits from cv. Ailsa Craig revealed low levels of bis(dihydrocaffeoyl)-spermine and spermidine, plus tris(dihydrocaffeoyl)spermine. No tris(dihydrocaffeoyl)spermidine could be detected, though two compounds perhaps representing *N*-caffeoyl-*N*′,*N*′′-bis-(dihydrocaffeoyl)spermidine isomers ($MH^+ = 636$, major fragments at *m*/*z* 474 and 163) were present in the same general area of the chromatogram.

Non-Solanaceous Species. No signs of any dihydrocaffeoyl polyamine conjugates were detected in single examples of *Arabidopsis thaliana* leaf or *Beta vulgaris* root extracts (data not shown).

At present, only a small proportion of plants have been subject to serious phytochemical analysis, and even here only certain specific subclasses of metabolites have usually been targeted. The past few years have, however, seen a growth of interest in the areas of functional genomics and systems biology, which serve to address issues such as how the genetic complement of an organism can affect its physiology and phenotype. This has resulted in an increased focus on metabolism and the compounds, as well as the enzymes and genes, that are involved (*5*−*10*). Metabolite-profiling methods are now being developed than can give a rapid qualitative and quantitative insight into the metabolite complement of a particular organism and how this may alter when conditions are changed.

In terms of our understanding of plant metabolism, these modern metabolomic techniques are having two important consequences. First, their relatively nontargeted nature provides the opportunity to examine chemical classes and biosynthetic pathways not normally studied, and second, a much greater range of plants can now be examined in depth, though of course some concentration on key crops and model species will no doubt still remain. In potato, we were able to identify a series of dihydrocaffeoyl polyamine conjugates present in significant amounts, and further investigation revealed that their biosynthetic capacity is present in several members of the Solanaceae. This is despite the fact that until now none of the metabolites had ever been identified from any of the species we examined and only one had ever been described from another plant source (*17*, *18*), though some of the related hydroxycinnamoyl compounds are a little more widely known (*29*, *31*). Our surprise discovery thus serves to illustrate the power of metabolomics. Given that at least some of these compounds have been suggested to possess beneficial biological activities (*17*, *19*), and that potato is a dietary staple for many humans, an awareness of these compounds could also have significant practical implications, though without detailed dose−response studies this currently remains hypothetical.

During our analyses, a number of more minor undescribed components were also observed. These had less informative mass spectra, and identification of these will require more extensive analysis centered on NMR. Developments in the application of combined LC/MS/NMR for single-step profiling are now well underway (*11*−*13*), offering the ability to make even such detailed analyses routine. Modern profiling techniques in metabolomic analysis are now reaching a position that should enable major breakthroughs to be made in both fundamental areas of functional genomics/systems biology and more applied areas of phytochemistry.

ABBREVIATIONS USED

LC/MS, liquid chromatography/mass spectrometry; DAD, diode array detector; ESI, electrospray ionization; DW, dry weight.

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