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ISOTHIOCYANATES AND THIOUREAS IN ENZYME HYDROLYSATES OF *TROPAEOLUM TUBEROSUM*

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Key Word Index—*Tropaeolum tuberosum*; Tropaeolaceae; chemotaxonomy; glucosinolate; isothiocyanate; thiourea; plant domestication.

Abstract—Analysis of the isothiocyanates arising from enzymatic hydrolysis of glucosinolate extracts of *Tropaeolum tuberosum* supports the assessment of two subspecies. Seeds, tubers, leaves and flowers of *T. tuberosum* subsp. *tuberosum* produced *p*-methoxybenzyl isothiocyanate. Subspecies *silvestre* produced benzyl-, 2-propyl- and 2-butylisothiocyanates. *N,N*-Di(4-methoxybenzyl)thiourea was detected in tuber extracts of subsp. *tuberosum* by HPLC.

INTRODUCTION

Tropaeolum tuberosum Ruiz & Pavon is an edible tuber producing cultigen of the Andes mountains. Wild plants as well as numerous cultivated clones are known and two subspecies, namely wild subsp. *silvestre* Sparre and cultivated subsp. *tuberosum*, have been distinguished [10] largely on the presence or absence of tubers. This taxonomic distinction appears questionable for classifying a feral tuber producing specimen collected in Peru.

Seeds of three samples of *T. tuberosum* collected in the wild in Peru were reported to produce benzyl- (**1**) and 2-propyl-isothiocyanates (**2**) as major constituents, and 2-butyl-isothiocyanate (**3**) in lesser amounts upon enzymatic hydrolysis of the glucosinolate-containing extract [8]. Glucosinolates of the cultigen have not been reported.

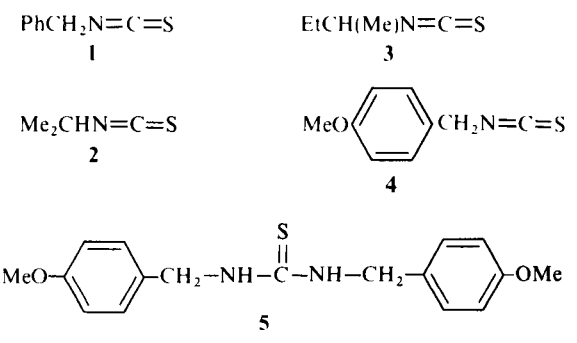
To determine the glucosinolates contained in *T. tuberosum* subsp. *tuberosum* we carried out a study of the isothiocyanates released in tubers, seeds, leaves and flowers of several clones, collected in Peru and propagated outdoors in Vancouver, Canada. Isothiocyanates of wild tuber producing material were studied in order to establish its taxonomic affinity. The distinction of a wild and a cultivated subspecies leads to speculation on the route of domestication of this important component of high-altitude agriculture in the Andes.

RESULTS AND DISCUSSION

Original tuber material of *T. tuberosum* subsp. *tuberosum* from four collections, and tubers from eight propagated clones showed identical isothiocyanate patterns. PC in two solvent systems of thiourea derivatives produced one spot corresponding in R_{ph} [9] and co-chromatographing with *p*-methoxybenzyl thiourea. Reverse phase HPLC showed a major peak (retention time, tr = 11.3 min) indistinguishable from that of benzyl-isothiocyanate but from MS and HNMR data the compound was identified as *p*-methoxybenzyl-isothiocyanate (**4**). A normal phase HPLC system resolved *p*-methoxybenzyl (tr = 3.8) and benzyl-isothiocyanates (tr = 3.4) and served as the method for the screening of all eight tuber samples and of seed, leaf and flower samples from one specimen, 'kello isaño', collected from Cuyo-cuyo, Peru. In all samples tested *p*-methoxybenzyl-isothiocyanate was the major component.

N,N-Di(4-methoxybenzyl)thiourea (**5**) was collected preparatively from isothiocyanate extracts of tubers of subsp. *tuberosum* by normal phase HPLC (tr = 17.0) and its identity determined by MS. This compound has been reported previously from glucosinolate-containing plants [2]. Its presence in extracts of *T. tuberosum* gives no new chemotaxonomic information, as the derivation of the compound from *p*-methoxybenzyl-isothiocyanate is obvious. Whether this type of thiourea is naturally formed in biological systems, or whether these compounds are artifacts of the extraction procedure has not been determined.

Seeds, tubers, leaves and flowers of 'kipa isaño', a wild type of *T. tuberosum* propagated from tubers, showed a pattern of isothiocyanates consistent with the previous report for this species [8]. Reverse phase HPLC of the isothiocyanate containing extracts revealed the presence of three isothiocyanates. Two peaks corresponded in tr and co-chromatographed with benzyl- and 2-propyl-



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isothiocyanate (tr = 10.1), respectively. The smaller peak (tr = 10.6) is probably 2-butyl-isothiocyanate. Mass spectral analysis of the benzyl peak collected preparatively confirmed its identity. PC of thiourea derivatives of isothiocyanates released from seeds and tubers revealed three components corresponding in R_{ph}s and co-chromatographing with benzyl-, 2-propyl- and 2-butyl-thioureas, respectively.

In both HPLC and PC, tubers showed relatively more of benzyl isothiocyanate than did seeds. 2-Butyl-isothiocyanate was not detected by PC from tuber samples and 2-propyl-isothiocyanate appeared as a minor constituent in PC and HPLC of tuber extracts. HPLC analysis of leaf and flower samples showed these plant parts to have intermediate amounts of benzyl isothiocyanate in relation to tubers and seeds.

The above analysis supports the assessment of two distinguishable subspecies within the species. *T. tuberosum* subsp. *tuberosum* is characterized by *p*-methoxybenzyl-glucosinolate. The material analysed by Kjaer *et al.* [8] and 'kipa isaño' are distinct from subsp. *tuberosum*. It is concluded that they are both examples of *T. tuberosum* subsp. *silvestre*, and that benzyl-, 2-propyl- and 2-butyl-isothiocyanates are characteristic of this subspecies.

An autotetraploid origin for the obligate cultivar probably starting with subsp. *silvestre* has been postulated [4], although a hybrid origin followed by segmental allopolyploidy with possibly *T. cochabambe* Buch. as a progenitor was also suggested. *T. tuberosum* subsp. *tuberosum* has a chromosome number of $2n = 52$ [4]. From root tips of 'kipa isaño' we have obtained a chromosome count of $2n = 42$ (unpublished results). Both subspecies had *ca* 95% stainable pollen indicating they are fertile.

If our analysis of one sample of 'kipa isaño' is representative of both the chemistry and ploidy level of subsp. *silvestre*, the autotetraploid origin of *T. tuberosum* subsp. *tuberosum* can be ruled out. If subsp. *tuberosum* did arise from subsp. *silvestre* it must have done so through hybridization with a taxon capable of producing *p*-methoxybenzyl-glucosinolate, such as *T. cochabambe* [8]. The chromosome number ($2n = 26$) of *T. cochabambe* [7] would preclude this species as a progenitor. Based on $x = 7$ known for some sections of the genus [10], subsp. *silvestre* ($2n = 42$) could be a hexaploid. Subspecies *tuberosum* could be an octaploid ($2n = 56$) derived from subsp. *silvestre* and a hypothetical diploid progenitor containing *p*-methoxybenzylglucosinolate. Subsequent loss of two pairs of chromosomes would leave it with a count of $2n = 52$.

Populations of wild or cultivated taxa variable in chemistry or ploidy level, may exist. If populations of subsp. *silvestre* with a chromosome number of $2n = 26$ do exist the suggestion that subsp. *tuberosum* arose from subsp. *silvestre* and *T. cochabambe* could still be supported.

Human intervention in the selection of cultivated varieties and in the selection of the glucosinolate chemistry of *T. tuberosum* subsp. *tuberosum* is a real possibility. *T. tuberosum* is a remnant of a primordial agricultural complex in the Andean highlands [5]. As well as continuing to provide an important food in localized areas it remains the subject of considerable folk belief [6]. The possible motivation for phytochemical selectivity, whether it be mediated by concerns for medicinal uses, tuber resistance to pathogen attack, flavour, or strictly cultural concepts, must be considered.

EXPERIMENTAL

Plant material. One tuber specimen of *T. tuberosum* subsp. *silvestre* (Johns No. 505) and two of subsp. *tuberosum* were collected by T.J. in Cuyo-cuyo, Department of Puno, Peru. Seeds of *T. tuberosum* subsp. *tuberosum* were obtained from Ing. H. Cortes Bravo of the Universidad Nacional del Cuzco, Peru. Tubers of three other recognizably different cultivars from farmers in Cuyo-cuyo and two from the market in Huancayo, Peru, were collected by Ing. A. Camino, Pontificia Universidad Catolica del Peru, Lima. Examples of all collections were propagated outdoors in Vancouver, Canada. Material collected within a 4-week period in Oct.–Nov. 1979 was refrigerated or frozen, and saved for analysis. Vouchers of *T. tuberosum* subsp. *silvestre* and subsp. *tuberosum* are deposited in the herbarium at UBC.

PC analysis of isothiocyanates. Tubers (15–40 g) and seeds (1–1.5 g) were extracted (2 ×) in a Waring blender with hot 70% MeOH. The filtrate was concd to dryness *in vacuo* and the residue disolved in citrate–phosphate buffer, pH 6.5. A crude myrosinase mixture containing ascorbic acid [3] was added and after 12 hr the reaction mixture was extrd with Et_2O and the extract treated with excess ethanolic NH_3 for 5 hr. The dried mixture was chromatographed (PC) on Whatman No. 1 paper in: (a) C_6H_6–EtOH–H_2O (5:1:2) [3] and (b) H_2O–satd $CHCl_3$ [9].

HPLC analysis. Tubers, flowers and leaves (5–40 g) and seeds (1–1.5 g) of both subspecies were extracted and hydrolysed as above. Additional tuber samples were hydrolysed by endogenous myrosinase [1]. The reaction mixtures were extracted with CH_2Cl_2 and the concd extract passed through a short column of Si gel and eluted with CH_2Cl_2. Samples for reverse phase HPLC were dried *in vacuo*, taken up in CH_3CN and filtered. A Varian Model 500 Liquid Chromatograph with a Varian Series 634 variable wavelength detector (245 nm) was used at ambient temp. For reverse phase chromatography a Micropak m-CH-10 analytical column with MeCN and H_2O as the mobile phase was used. Samples were injected at 35% MeCN and run at a flow rate of 1 ml/min over a gradient of 10%/min to 70% CH_3CN. Prep. chromatography was carried out by a similar method using a m-CH-10 preparative column and a flow rate of 2 ml/min. For normal phase chromatography a Micropak NH_2-10 column with CH_2Cl_2–*iso*-octane (3:7) as the mobile phase provided the best results. Standards were co-chromatographed with all samples where possible. For preparative work fractions from repeated runs were pooled, extracted into CH_2Cl_2 and concd *in vacuo*. Identity of samples prepared in this way were confirmed by the thiourea-PC method described above, and by MS and/or ^{1}H NMR.

MS analysis. MS of benzyl and *p*-methoxybenzyl isothiocyanates and of *N,N*-Di(4-methoxybenzyl) thiourea was carried out on an Atlas MAT (Bremen) CH4-B Mass Spectrometer. The *m/z* values corresponded with published data [2].

^{1}H NMR of p-methoxybenzyl isothiocyanate. ^{1}H NMR (80 MHz, $CDCl_3$, TMS): δ 3.80 (3 H, *s*, MeO), 4.60 (2 H, *s*, CH_2), 7.04 (4 H, *m*, $J = 8.8$ Hz, C-2, C-3).

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