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# Purine alkaloids in *Paullinia*

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**Abstract**

Among the few purine alkaloid-containing genera consumed as stimulants, *Paullinia* is the least investigated with respect to both chemotaxonomy and within-the-plant allocation of caffeine and its allies. Since purine alkaloids (PuA) have been proved to be valuable marker compounds in chemotaxonomy, 34 species of *Paullinia* and related genera were screened for them, but only one, *P. pachycarpa*, was positive in addition to the already known *P. cupana* and *P. yoco*. The PuA allocation in *P. pachycarpa* was examined and found to be restricted to theobromine in the stem, leaves and flowers. Moreover, the theobromine concentration in the stem cortex increased significantly towards the base of the plant. Since the stem cortex of *P. yoco* is traditionally used by the natives of Colombia and Ecuador to prepare a caffeine-rich beverage, we suspected that within the genus *Paullinia* the PuA are preferentially allocated to the older parts of the stem and not to young shoots like e.g., in the coffee plant (*Coffea* spp.). Indeed, the axis (greenhouse) of *P. cupana* (guaraná), known for its caffeine-rich seeds, exhibited a basipetal PuA gradient (0.005–0.145%). Moreover, the analysis of young cortex samples (herbarium) and of one piece of old stem (museum collection) revealed the same for *P. yoco*, even though we found much less (0.5 vs 2.5%) caffeine in the old cortex as compared to the only two analyses in 1926 of similar material. However, this discrepancy may be explained by the high variability of the PuA pattern we detected among *yoco*, the diversity of which the Indians take advantage.


*Keywords:* *Paullinia cupana*; *P. pachycarpa*; *P. yoco*; Sapindaceae; Guaraná; Yoco; HPLC; Purine alkaloids; Caffeine; Cortex

## 1. Introduction

Worldwide, six caffeine-containing genera are used to prepare pleasant stimulants, namely *Coffea*, *Camellia*, *Theobroma*, *Cola*, *Ilex*, and *Paullinia*. Whereas the first three taxa have been well studied regarding the occurrence and within-the-plant distribution of purine alkaloids (PuA), *Coffea* (Charrier and Berthaud, 1975), *Camellia* (Zheng et al., 2002), *Theobroma* (Hammerstone et al., 1994), the remaining three, i.e., *Cola*, *Ilex*, and *Paullinia*, are poorly investigated in this respect, most likely because of their lesser economic importance and/or species richness. However, since PuA are useful in chemosystematics (Hammerstone et al., 1994) and also have both ecological (Kretschmar and Baumann, 1999; Hollingsworth et al., 2002) and ethnobotanical (Beck, 1990) significance, it was an intriguing task to tackle the lesser known genera mentioned above. Here, we report on the extension of our earlier investigations into the genus *Paullinia*.

*Paullinia* belongs to the Sapindaceae and comprises ca. 180 species, all neotropical lianas with the exception of *P. pinnata* occurring also in the African tropics. So far only two species have been reported to contain PuA: *P. cupana* H.B.K. and *P. yoco* Schultes & Killip. Some years ago we made a detailed analysis (Baumann et al., 1995) of the guaraná fruit (*P. cupana* H.B.K. var. *sorbilis* (Mart.) Ducke), the caffeine-rich seeds of which are traditionally used by the natives in the central Amazon region of Brazil to prepare a stimulating beverage (Henman, 1982). The concern of the present study, however, is to investigate the liana *Paullinia yoco*, a caffeine-containing plant of which only the bark serves for the preparation of a stimulant (Schultes, 1987). The traditional and geographically very restricted use by the Indians consists of rasping the stem cortex and either squeezing or simply immersing the resulting mass into cold water to prepare a caffeine-rich drink. The tribes

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using *yoco* live in southern Colombia (Col) or/and in northeastern Ecuador (Ec) and are the following: Coreguajes (Col), Ingas (Col), Kofáns (Col/Ec), Sionas (Col/Ec), and Secoyas (Ec) (Schultes, 1942; Vickers and Plowman, 1984; Paz y Miño et al., 1991; Garcá-Barriga, 1975; Schultes and Raffauf, 1990). It is the general custom of these natives to eat nothing until noontime. Instead, *yoco* is taken each morning between five and six o'clock, to allay all sensations of hunger for at least 3 h and to supply muscular stimulation (Schultes, 1942).

The Indians recognize and classify various 'types' of *yoco*: *yoco blanco*, *yoco verde*, *huarmy yoco*, *taruca yoco*, *yagé yoco*, and *canaguicho yoco* among others (Schultes, 1986; 1987). Frequently, these designations were put on the specimen labels by the collectors. It is most interesting what Schultes wrote about the ability of the indigenous people to discriminate between the various types of *yoco*: "They (the Indians) are able to identify these kinds by name often at a considerable distance and without touching, tasting, cutting, or smelling any part of the plant" (Schultes, 1987), or "The botanists who have gathered the names and have associated them with collections of *yoco* cannot offer distinguishing characters" (Schultes, 1986). Even though some of the names indicate the use of *yoco* in combination with a second plant species, e.g. *yagé yoco* (*yagé* means *Banisteriopsis* spp.), or *canaguicho yoco* (*canaguicho* refers to *Mauritia flexuosa*), there doesn't seem to be any scientific rationale explaining such a wide Indian *yoco* diversity.

In 1906 Rafael Zerda-Bayón reported for the first time on the use of *P. yoco* (Schultes, 1942; 1986). Apparently, Florent Claes initiated the scientific exploration of this plant species in the remote region of Caquetá, Colombia, by collecting in 1925 a large quantity of both stem material for chemical investigations and flowering specimens for taxonomic studies. The stems were chemically analyzed in Brussels as well as in Paris and the cortex was found to contain caffeine in the range of 2 to 2.75% dry wt. (Michiels and Denis, 1926a; 1926b; Rouhier and Perrot, 1926). The Belgian taxonomist Emile Auguste De Wildeman (1866–1947), director of the botanical garden of Belgium (1912–1931), falsely assigned the collected specimens to *Paullinia scarlatina* (Michiels and Denis, 1926a), a species living exclusively in Central America (Radlkofer, 1933). Finally, in 1942 Schultes identified *P. yoco* as a new species (Schultes, 1942). Today, worldwide ca. 40 collections of *P. yoco* exist, but unfortunately mature fruits, which are indispensable for systematics in this genus, have never been described. Thus, the systematic position of this species remains questionable (Beck, 1991).

Considering that so far only the above-mentioned two *Paullinia* species have been reported to contain PuA, we decided to screen a large number of *Paullinia* specimens for the occurrence of PuA. Additionally, we tried to shed some light on the unusual allocation of caffeine to the stem's periphery of *P. yoco*, and finally, it was an intriguing task to look for a possible correlation between the above-mentioned Indian designations for *yoco* and the PuA pattern found during our analyses.

## 2. Results and discussion

### 2.1. Screening for PuA

Among the 34 species (90 specimens) tested we found besides *Paullinia cupana* and *P. yoco*, already known as PuA-rich, only one new alkaloid-bearing species, namely *P. pachycarpa*. Similarly, Michiels and Denis (1926) had screened 37 *Paullinia* species without a positive hit, whereby 28 were different from ours. Because of the scarce material, the authors considered their results as preliminary.

Even though PuA have been shown to provide distinct markers on various taxonomic levels, cf. *Theobroma* (Hammerstone et al., 1994), *Ilex* (Zographos and Baumann, unpublished), and the *Citrus* clade (Kretschmar and Baumann, 1999; Stutz and Baumann, unpublished), the situation of *Paullinia* is not as clear. Unfortunately, a molecular-based phylogenetic analysis of Paullinieae is lacking and the systematic relationship between the three PuA-containing species therefore remains unresolved. Based on fruit characters, Radlkofer (1895, 1933) placed *P. cupana* and *P. pachycarpa* in different sections, namely *Pleurotoechus* and *Pachytoechus*, respectively. When Schultes (1942) described *P. yoco* as a new species, he included it in the section *Enourea*, most likely arbitrarily, since no fruits were available.

### 2.2. Detailed analysis of Paullinia pachycarpa

Our analyses revealed theobromine as the only component in all PuA-containing plant organs (Table 2). Amazingly, young tender leaflets (length 13–55, width 3–20 mm) were virtually alkaloid-free, whereas adult leaflets (length 65–160, width 25–75 mm) contained well-measurable alkaloid concentrations, i.e., a median value of 0.025% (dry wt.). Though on a much lower level, this alkaloid allocation is inverse to that found in other PuA-bearing species such as *Coffea arabica* (Frischknecht et al., 1986) or *Camellia sinensis* (Fujimori et al., 1991; Ito and Ashihara, 1999). We conclude that the function of theobromine in *P. pachycarpa* may not be primarily linked to chemical defense. Based upon the optimal defense theory claiming that plant organs accumulate protective phytochemicals in direct proportion to their risk of predation (Rhoades, 1979), phytochemicals other than PuA must act as key defense

compounds. However, preliminary rapid tests on saponins (shaking in water) and cyanogenic glycosides (commercially available test stripes) were negative. The median values (% dry wt.) for theobromine of the axis cortex, stem cortex and stem wood were 0.006, 0.018 and 0.001, respectively. In all seven individuals analyzed, the theobromine concentration in the stem cortex was at least ten times higher than in the stem wood and three to four times higher than in the axis cortex (at the top of the plant) of the same individual. Similarly, in *P. cupana* the cortex theobromine content increased by a factor of 18 from the top (axis) toward the base (stem), i.e., from 0.005 to 0.092 (Table 2). We may conclude that in both *P. cupana* and *P. pachycarpa* the PuA content of the cortex is positively correlated with the age of the stem.

In the flowers of *P. pachycarpa* only traces of theobromine could be detected. The almost mature fruits, i.e., pericarp and seeds, are virtually alkaloid free. This contrasts with the situation met in *P. cupana* and *P. yoco*. Both accumulate considerable PuA amounts in flowers (Table 2), and *P. cupana* is known to furnish seeds and pericarp with high alkaloid concentrations (Baumann et al., 1995).

### 2.3. *Paullinia yoco*

To determine the PuA content of *Paullinia yoco*, leaflet samples of 26 specimens and axis cortex samples of 23 specimens, respectively, were analyzed (Table 1). Even though a possible non-uniform alkaloid distribution within the leaflet was met with selective sampling, i.e., always near the margin, approximately in the middle between base and tip of the lamina, the related values (% dry wt.) of theobromine and caffeine extremely varied, that is 0.000–0.438 and 0.000–1.308, respectively (Table 3). In this context it has to be mentioned that PuA allocation within the lamina of the entire leaves of both *Coffea arabica* and *Ilex paraguariensis* (Wenger and Baumann, unpublished) was found to be gradient-like, considerably increasing from the central vein towards the margin, very similar to the nicotine distribution within the tobacco leaf (Burton et al., 1992). To our knowledge related analyses on compound leaves have not been done so far. Whatever the distribution in the *yoco* leaf may look like, it will not fully explain the high variation apparent in Table 3. It could well be that, besides the 'genotype' (see below), the collecting practices contributed to it. As outlined in 'Experimental' we do not know, whether the material from the various herbaria got in contact with EtOH while collected. To get a rough estimate of a related and possible loss of PuA, we simulated the application of EtOH using fresh leaves of *P. cupana* as outlined in 'Experimental'. Expectedly, they lost 28 to 86 and 57 to almost 100% of theobromine and caffeine (minor alkaloid in leaves of this species), respectively, most likely by diffusion into the newspaper. In any case, however, the residual alkaloid found was sufficient to positively identify the material as alkaloid-bearing, a condition necessary to exclude falsely assigned herbarium samples (see below). In conclusion, the original material of *P. yoco* might have contained in some samples higher PuA values, but the complete absence of alkaloids would mean that the sample was naturally alkaloid-free.

In *P. yoco* theobromine and caffeine co-occurred at about the same concentration range (Tables 2 and 3), whereas minor PuA were absent—a situation quite unique and thus characteristic for this species. Two specimens (Gentry et al. 56588 and Palacios 4048; Table 1) were found to be entirely alkaloid-free and therefore do not belong to *P. yoco*, and the specimen of Vasquez & Jaramillo 13545 (Table 1) can be assigned to *P. cupana* due to the additional note "guaraná" on the specimen label and its location near Iquitos, where *P. cupana* is cultivated (cortex: tb 0.08, ca 0.02; leaves: tb 0.12, ca. 0.00). In the remaining 23 specimens of *P. yoco* and related to the individual leaflets, either theobromine or caffeine was predominant. This is also true for the axis cortex, the overall values (dry wt.) of which considerably varied like those of the leaflet mentioned above, that is 0.005–0.185 and 0.000–0.525 for theobromine and caffeine, respectively (Table 3). The highest maximum values (Table 3) of both alkaloids were found in the leaflets (0.438 and 1.308), highest median values (Table 2), however, in the cortices (0.048 and 0.100).

Luckily, we had the opportunity to re-examine the traditionally used *yoco* stem material with a diameter of 4 cm, the cortex and wood of which had been reported in 1926 to contain as much as 2 to 2.73 and 0.54% caffeine and theobromine, respectively (Michiels and Denis, 1926a; 1926b). From our studies on the behavior of theobromine and caffeine in the cortices of both *P. cupana* and *P. pachycarpa* showing a significant increase towards the base of the plant (Table 2), we must assume that the same positive, stem age-dependent correlation operates also in *P. yoco*. Since we found in the young cortex of *yoco* a caffeine content between 0.0 and 0.5% (median value 0.1%), one might expect at the stem base the amazingly high concentrations mentioned above. However, as presented in Fig. 1, our values were much lower. The highest caffeine content we found was that of the bark (almost 0.8%), whereas the cortex had only about 0.45%. The wood, however, contained remarkable 0.28%. Theobromine was present in a surprisingly stable ratio to caffeine of about 1:67 throughout the entire radius. Since we assume that our sample is not identical to the one analyzed more than 75 years ago, we may explain the discrepancy between the caffeine contents only by phytochemical variability among the *yocos* as possibly suggested by the values of Table 3. Clearly, fresh stem material of various *yoco* provenances will be

Table 1
Specimens analyzed

| Taxa | Collection | Herbarium | Method | Analyzed material |
|---|---|---|---|---|
| *Cardiospermum halicacabum* | W&I 000710-1/3 | MOL | m | l |
| *Cardiospermum halicacabum* | W&I 000710-1/3 | MOL | h | ly |
| *Paullinia* aff. *alsmithii* | W&I 000409-1/1 | MOL | m | l |
| *Paullinia* aff. *cuneata* | W&I 000615-1/3 | MOL | m | l |
| *Paullinia* aff. *cuneata* | W&I 000615-1/3 | MOL | e | l |
| *Paullinia* aff. *cuneata* | W&I 000615-1/3 | MOL | h | ly |
| *Paullinia* aff. *pterophylla* | Schunke 9401 | F | e | l |
| *Paullinia* aff. *subauriculata* | W&I 000308-1/1 | MOL | m | l |
| *Paullinia carpopodea* | Dusén 5012 | M | e | l |
| *Paullinia carpopodea* | Dusén 8051 | M | e | l |
| *Paullinia carpopodea* | Sellow 449 | M | e | l |
| *Paullinia carpopodea* | Schwacke 7353 | M | e | l |
| *Paullinia clathrata* | W&I 000527-1/5 | MOL | m | l |
| *Paullinia clathrata* | W&I 000611-1/5 | MOL | m | l |
| *Paullinia clathrata* | W&I 000611-1/5 | MOL | e | l |
| *Paullinia clathrata* | W&I 000611-1/5 | MOL | h | ly |
| *Paullinia clavigera* | W&I 000604-2/3 | MOL | m | l |
| *Paullinia clavigera* | W&I 000604-2/4 | MOL | m | l |
| *Paullinia costata* | Schenk 756 | M | e | l |
| *Paullinia costata* | Türkheim 7737 | M | e | l |
| *Paullinia cupana* | W&I 000604-1/1 | MOL | m | l |
| *Paullinia elegans* | W&I 000527-1/7 | MOL | h | ly |
| *Paullinia elegans* | W&I 000717-1/1 | MOL | m | l |
| *Paullinia elegans* ssp. *neglecta* | W&I 000307-1/2 | MOL | m | l |
| *Paullinia eriocarpa* | W&I 000613-1/5 | MOL | m | l |
| *Paullinia eriocarpa* | W&I 000613-1/5 | MOL | e | l |
| *Paullinia eriocarpa* | W&I 000613-1/5 | MOL | h | ly |
| *Paullinia faginea* | W&I 000615-1/1 | MOL | m | l |
| *Paullinia faginea* | W&I 000615-1/2 | MOL | m | l |
| *Paullinia faginea* | W&I 000615-1/2 | MOL | e | l |
| *Paullinia ferruginea* | unknown | M | e | l |
| *Paullinia grandifolia* | W&I 000401-1/1 | MOL | m | l |
| *Paullinia grandifolia* | W&I 000611-1/1 | MOL | m | l |
| *Paullinia hystrix* | W&I 000531-1/5 | MOL | h | ly |
| *Paullinia ingifolia* | Schwacke 4003 | M | e | l |
| *Paullinia itayensis* | W&I 000611-1/4 | MOL | m | l |
| *Paullinia itayensis* | W&I 000611-1/4 | MOL | e | l |
| *Paullinia obovata* ssp. *flava* | W&I 000527-1/4 | MOL | m | l |
| *Paullinia obovata* ssp. *obovata* | W&I 000331-2/1 | MOL | m | l |
| *Paullinia obovata* ssp. *obovata* | W&I 000718-2/1 | MOL | h | ly |
| *Paullinia obovata* ssp. *subrotunda* | W&I 000707-1/1 | MOL | h | ly |
| *Paullinia obovata* ssp. *subrotunda* | W&I 000315-2/1 | MOL | m | l |
| *Paullinia pachycarpa* | W&I 000307-1/1 | MOL | m | flb, l |
| *Paullinia pinnata* | Zoro s.n. | Z | a | l |
| *Paullinia pterophylla* | Karsten s.n. | W | e | l |
| *Paullinia seminuda* | Hoehne 8829 | M | e | l |
| *Paullinia seminuda* | Schenk 1166 | M | e | l |
| *Paullinia seminuda* | Schwacke 11468 | M | e | l |
| *Paullinia sphaerocarpa* | W&I 000529-1/1 | MOL | m | l |
| *Paullinia sphaerocarpa* | W&I 000604-1/2 | MOL | m | l |
| *Paullinia spicata* | W&I 000531-1/3 | MOL | m | l |
| *Paullinia spicata* | W&I 000604-2/1 | MOL | m | l |
| *Paullinia spicata* | W&I 000604-2/2 | MOL | m | l |
| *Paullinia stenopetala* | Mélinon 1862 | M | e | l |
| *Paullinia yoco* | Ceron & Hurtado 3955 | MO | e | l, ac |
| *Paullinia yoco* | Ceron 150 | NY | e | l, ac |
| *Paullinia yoco* | Ceron 327 | NY | e | l, ac |
| *Paullinia yoco* | Claes 23 | BR | e | l, ac |
| *Paullinia yoco* | Claes 24 | BR | e | l, ac |
| *Paullinia yoco* | Claes 30 | BR | e | l, ac |

(continued)

Table 1 (*continued*)

| Taxa | Collection | Herbarium | Method | Analyzed material |
|---|---|---|---|---|
| *Paullinia yoco* | Gentry et al. 56588 | MO | e | l, ac |
| *Paullinia yoco* | King 21 | F | e | l |
| *Paullinia yoco* | King 468 | F | e | l |
| *Paullinia yoco* | Klug 1930 | NY | e | l, ac |
| *Paullinia yoco* | Klug 1930 | W | e | l, ac |
| *Paullinia yoco* | Klug 1933 | NY | e | l, ac |
| *Paullinia yoco* | Klug 1935 | NY | e | flb, l, ac |
| *Paullinia yoco* | Klug 1935 | W | e | l, ac |
| *Paullinia yoco* | Klug 1937 | NY | e | l, ac |
| *Paullinia yoco* | Klug 1946 | NY | e | l, ac |
| *Paullinia yoco* | Klug 1947 | NY | e | l, ac |
| *Paullinia yoco* | Lescure 2104 | NY | e | l, ac |
| *Paullinia yoco* | Pinkley 380 | NY | e | l, ac |
| *Paullinia yoco* | Schultes s.n. A | NY | e | l, ac |
| *Paullinia yoco* | Schultes s.n. B | NY | e | l, ac |
| *Paullinia yoco* | Schultes s.n. C | NY | e | l, ac |
| *Paullinia yoco* | Vasquez & Jaramillo 13545 | MO | e | l, ac |
| *Paullinia yoco* | Vickers 109 | F | e | l |
| *Paullinia yoco* cf. | Neill et al. 7351 | NY | e | l, ac |
| *Paullinia yoco* cf. | Palacios 4048 | MO | e | l, ac |
| *Paullinia* sp. | W&I 000409-1/1 | MOL | h | ly |
| *Paullinia* sp. | W&I 000606-1/1 | MOL | m | l |
| *Paullinia* sp. | W&I 000606-1/1 | MOL | h | ly |
| *Serjania altissima* | W&I 000719-1/7 | MOL | h | ly |
| *Serjania grammatophora* | W&I 000719-1/8 | MOL | m | l |
| *Serjania inflata* | W&I 000724-2/2 | MOL | h | ly |
| *Serjania rubicaulis* | W&I 000314-2/1 | MOL | m | l |
| *Thinouia* sp. | W&I 000710-1/2 | MOL | m | l |
| *Urvillea ulmacea* | W&I 000705-1/1 | MOL | h | ly |
| *Urvillea ulmacea* | W&I 000703-1/6 | MOL | m | l |

ac = cortex of young axis; flb = floral bud; l = adult leaflet; ly = young leaflet; m = MASE; e = extrelut; h = 0,1 N HCl directly injected; W&I = Weckerle and Igersheim.

necessary to fully resolve the '*yoco* mystery'. Nevertheless, we are faced with an unusual, basipetal allocation of PuA within the cortex, and possibly also within the wood. This unexpected phytochemical allocation characteristic of the genus *Paullinia* calls for a plant physiological explanation. Caffeine is known to pass easily through all kinds of biological membranes and barriers, presumably due to its dual (hydrophilic and lipophilic) character, which also explains the rapid CNS-stimulating effect after consumption of a caffeine-containing beverage (Baumann and Seitz, 1992). However, in the 'purine alkaloid plants' a large fraction of caffeine is usually fixed by complexation (in the vacuole) with phenols, such as chlorogenic acids in coffee (Mösli Waldhauser and Baumann, 1996). In the seeds of *P. cupana* catechins act as the complexing agent (Marx, 1990), and it is assumed that they exert this role in other plant parts as well as in other species of this genus. Since caffeine passively diffuses within the plant, one should expect a distribution similar to that of the complexor. Indeed, within the coffee leaf we found a non-uniform distribution of both caffeine and chlorogenic acids with a distinct accumulation at the margins (Wenger and Baumann, unpublished). Undoubtedly, this 'phytochemical architecture' is in favor of a site-directed chemical defense: the leaf edge is a preferential site of attack of e.g., phytophagous insects. In the case of *Paullinia* we have to postulate high concentrations of polyphenolics in the old stem cortex leading to a considerable accumulation of caffeine possibly exceeding its solubility. In any case, this characteristic allocation of the well-diffusible caffeine in *Paullinia* is a further example of the surprising strategies evolved by higher plants to establish an optimal 'phytochemical architecture'.

### 2.4. *Yoco* diversity

According to Schultes (1986) the Indians recognize and classify various 'types' of *yoco* (cf. 'Introduction'). We tried to find a correlation between PuA and the *yoco* type indicated on the label of the specimen. Very strikingly, highest median values (% dry wt.) for theobromine and caffeine in leaflets and cortices (0.263 and 1.043; 0.110 and 0.167%, respectively) were found in *huarmy yoco* (Table 3). This coincides with the note on

the specimen label by Klug 1935 describing *huarmy yoco* as the strongest type. The specimens of *yoco blanco* had a highly variable PuA content in both leaflets and cortices, but with a median value of caffeine higher in the cortex, this in contrast to *huarmy yoco* (Table 3). The median values of the *yoco verde* leaflets were between those of *huarmy yoco* and *yoco blanco*, whereas those of the axis cortex were relatively low. No PuA could be detected in the leaflets of *yagé yoco* and *canaguicho yoco*, and their cortices exhibited a low content only. *Taruca yoco* shows intermediate values in the leaflets as well as in the cortex. The results clearly indicate that at least some of the indigenous *yoco* types are chemovars. Again, analyses of fresh plant material collected on the spot will be needed to fully explain this most intriguing enigma raised by Schultes (1986). Finally, we should mention that the isolation of caffeine from the stem of *Banisteriopsis inebrians* by O'Connell (1969) can be explained by Indian nomenclature: *yagé yoco* means the *yoco* used together with *yagé*, i.e. with *Banisteriopsis* spp., in *ayahuasca*. Thus, most probably, O'Connell (1969) analyzed *Paullinia yoco* type *yagé yoco*, instead of *Banisteriopsis*!

Table 2
Median values of purine alkaloid content (% dry wt.) in different plant organs of *Paullinia pachycarpa*, *P. cupana* and *P. yoco*

| | Theobromine | Theophylline | Caffeine |
|---|---|---|---|
| *Paullinia pachycarpa* ($n=7$) | | | |
| Young leaflet | Trace | * | * |
| Adult leaflet | 0.025 | * | * |
| Axis cortex | 0.006 | * | * |
| Stem cortex | 0.018 | * | * |
| Stem wood | 0.001 | * | * |
| Floral bud° | Trace | * | * |
| Pericarp° | * | * | * |
| Seed (cotyl.)° | * | * | * |
| *Paullinia cupana* ($n=6$) | | | |
| Young leaflet | 1.263 | * | 0.003 |
| Adult leaflet | 0.028 | * | * |
| Axis cortex | 0.005 | * | * |
| Stem cortex | 0.092 | * | 0.053 |
| Floral bud° | 0.139 | * | 0.021 |
| Pericarp | 0.203a | 0.001a | 0.02a |
| Seed (cotyl.) | 0.015a | 0.007a | 4.28a |
| *Paullinia yoco* | | | |
| Adult leaflet ($n=23$) | 0.030 | * | 0.025 |
| Axis cortex ($n=20$) | 0.048 | * | 0.100 |
| Stem cortex° | 0.009 | * | 0.449 |
| Stem wood° | 0.006 | * | 0.281 |
| Floral bud° | 0.053 | * | 0.186 |

* = not detectable.
° $n=1$ (several floral buds from one plant).
a = from Baumann et al. (1995).

## 3. Experimental

### 3.1. Plant material

Herbarium material of various species of *Paullinia* as well as of the closely related genera *Cardiospermum*, *Serjania*, *Thinouia*, and *Urvillea* was screened for the presence of PuA (Table 1). Small samples (5–30 mg) from either adult or young leaves, as indicated, were removed with a scalpel or a pair of tweezers. Unfortunately, we do not know whether the herbarium specimens collected by others had been soaked in ethanol (EtOH) immediately after collection, a practice suspected of removing part of the alkaloids (see 'PuA analysis'). The specimens collected by Weckerle and Igersheim were occasionally and superficially treated with 70% EtOH if necessary, e.g., moldy spots on fruits.

The *Paullinia* species identified to contain PuA, namely *P. cupana*, *P. pachycarpa* and *P. yoco*, were further analyzed preferentially regarding the allocation of PuA in leaves and stem cortex. Of *P. cupana* fresh material was taken from several-year-old individuals ($n=6$) kept in our greenhouse. *P. pachycarpa* was collected in Peru ($n=7$), whereas for the examination of *P. yoco* only herbarium specimens were available (Table 1). Of *P. cupana* and *P. pachycarpa* the following plant organs were analyzed: young leaflets, adult leaflets, cortex of the young axis collected 10–40 cm below the shoot apex, wood and cortex of the stem taken at the stem base (∅ 2.5–4.0 cm, ca. 10 cm above ground for *P. cupana*; ∅ 2.0–6.7 cm, ca. 10–50 cm above ground for *P. pachycarpa*). Additionally twelve floral buds (just before anthesis) and a single, almost mature fruit (pericarp and seed) of one individual of *P. pachycarpa* as well as three floral buds of one individual of *P. cupana* were analyzed. Fresh material was dried at 62 °C to constant weight. From *P. yoco,* besides the above-mentioned leaf samples small cortex probes of the young axis (2–10 mg) and three floral buds of one specimen (Klug, 1935) were analyzed (Table 1). Very fortunately, we received from the museum of the University of Paris (Musée de Matière Médicale, Collection du Laboratoire de Pharmacognosie de la Faculté de Pharmacie de Paris) a generous gift of a *yoco* stem section ($r=2$ cm). Most likely, this material can be traced back to that collected by Claes in 1925 and analyzed one year later by the Belgian and French phytochemists mentioned in the 'Introduction' (Michiels and Denis, 1926a, 1926b; Rouhier and Perrot, 1926). A part (ca. 2 mm in thickness) of this section was sliced longitudinally into 7 portions (5 of wood and 2 of cortex) with the following weights (mg; from the center to the periphery): 116, 132, 115, 89, 122, 193, 134. The outermost layer, a thin, dead outer bark, was scratched off with a knife and yielded portion 8 with 45 mg.

### 3.2. PuA analysis

Two different extraction methods, MASE and 'Extrelut®' were used, whereas the former was only applied for PuA screening of the specimens.

For MASE (microwave-assisted sample extraction) the dried leaf probes were moistened in a 5-ml screw cap vial for 30 min by pipetting 20 μl $H_2O$. Then, 1 ml $CH_2Cl_2$ was added and the vial tightly closed. After the exposure to microwave, 2× for 3 min with an in-between-cooling in tap water, 900 μl of the extract were transferred into a HPLC vial, and the solvent was removed by a stream of $N_2$. The residue was dissolved in 500 μl 8% MeOH and analyzed by HPLC.

In the 'Extrelut®' method the sample material was either homogenized in a mill (large probes) or crushed with a pair of tweezers (small probes) and extracted with 1 or 5 ml 0.1N HCl in a snap-cap vial for 30 min at 50 °C. An aliquot of 500 μl of the extract was applied onto a Kieselgur column (Extrelut®, Merck, Darmstadt, Germany) and, after 5 min, elution was carried out with 5 ml $CH_2Cl_2$. The solvent was removed by a stream of $N_2$, and the residue dissolved in 500 μl 8% MeOH. Aliquots of 50–100 μl were analyzed by HPLC.

HPLC. Separation was performed on a Nucleosil 100-5 ODS (ChromCart, Macherey-Nagel) column (5 μm; 4×125 mm; precolumn 4×8 mm) with $H_2O$ [A] and MeOH [B], both with 1% THF, at a total flow rate of 1 ml $min^{-1}$ and by the following gradient (%B over A): 0–10 min (8–25). Parameters were controlled by a Gynkotek (Dionex, Olten, Switzerland) liquid chromatograph equipped with a diode array detector set at 272 nm. Peak identification was achieved by comparing UV spectrum (library established under separating conditions) and retention time of authentic standards. The retention times (min) were as follows: theobromine (4.0), theophylline (6.5), caffeine (9.0). The detection limit was $10^{-6}$% (dry wt.). Samples with lower concentration were designated as virtually alkaloid-free.

To simulate the many collectors' practice of applying EtOH to freshly collected plant specimens (see 'Plant Material') and to estimate a possible loss of leaflet PuA, the following experiment was set up twice with *P. cupana*: a 'sandwich' consisting of one fully expanded leaflet, one newspaper sheet moistened with 70% EtOH, and one fresh young leaflet was wrapped into wettened (70% EtOH) newspaper and kept for 3 weeks in a plastic bag at 25 °C (greenhouse conditions). Before and

Table 3
Purine alkaloid content (% dry wt.) in different *Paullinia yoco* specimens

| Taxon | Collector | *Yoco* 'type' | Plant material | Theobromine | Caffeine |
|---|---|---|---|---|---|
| *Paullinia yoco* | Ceron 327 | *totoa* (white) *yoco* | Adult leaflet | 0.030 | 0.523 |
| | | | Axis cortex | 0.079 | 0.140 |
| | Klug 1933 | *blanco yoco* | Adult leaflet | 0.199 | 0.674 |
| | | | Axis cortex | 0.049 | 0.525 |
| | Pinkley 380 | *totoa yoco* | Adult leaflet | 0.000 | 0.000 |
| | | | Axis cortex | 0.027 | 0.027 |
| | Schultes s.n. A | *blanco yoco* | Adult leaflet | 0.126 | 0.003 |
| | | | Axis cortex | 0.042 | 0.046 |
| | Schultes s.n. B | *blanco yoco* | Adult leaflet | 0.135 | 0.008 |
| | | | Axis cortex | 0.107 | 0.099 |
| | Schultes s.n. C | *blanco yoco* | Adult leaflet | 0.028 | 0.025 |
| | | | Axis cortex | 0.049 | 0.062 |
| | | median | Adult leaflet | 0.078 | 0.016 |
| | | | Axis cortex | 0.049 | 0.081 |
| | Klug 1930 | *verde yoco* | Adult leaflet | 0.296 | 0.000 |
| | | | Axis cortex | 0.038 | 0.005 |
| | Klug 1930 | *verde yoco* | Adult leaflet | 0.018 | 0.216 |
| | | | Axis cortex | 0.064 | 0.016 |
| | | median | Adult leaflet | 0.157 | 0.108 |
| | | | Axis cortex | 0.051 | 0.010 |
| | Klug 1935 | *huarmi* (strongest) *yoco* | Adult leaflet | 0.087 | 1.308 |
| | | | Axis cortex | 0.035 | 0.161 |
| | Klug 1935 | *huarmi yoco* | Adult leaflet | 0.438 | 0.778 |
| | | | Axis cortex | 0.185 | 0.174 |
| | | median | Adult leaflet | 0.263 | 1.043 |
| | | | Axis cortex | 0.110 | 0.167 |
| | Klug 1946 | *yagé yoco* | Adult leaflet | 0.000 | 0.000 |
| | | | Axis cortex | 0.005 | 0.000 |
| | Klug 1947 | *canagiucho yoco* | Adult leaflet | 0.000 | 0.000 |
| | | | Axis cortex | 0.009 | 0.038 |
| | Klug 1937 | *taruca* (poisonous) *yoco* | Adult leaflet | 0.278 | 0.030 |
| | | | Axis cortex | 0.130 | 0.045 |

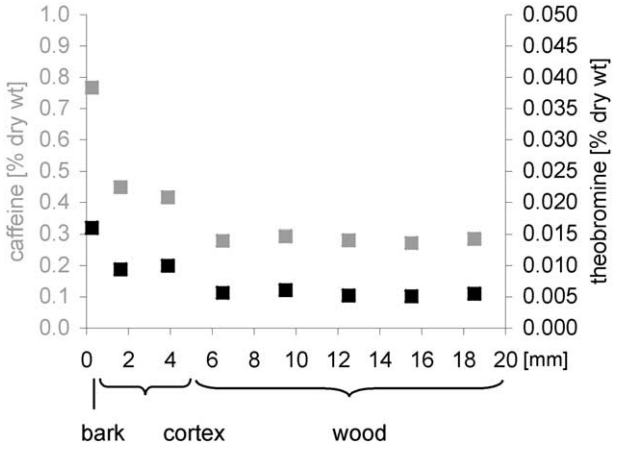


Fig. 1. Concentration of caffeine and theobromine [% dry wt.] in bark, cortex and wood of a stem section (radius 20 mm) of *Paullinia yoco*.

after this exposure, one probe of ca. 10 mg was taken for HPLC analysis from one and the other leaflet half, respectively, always close to the leaflet margin approximately in the middle between lamina base and tip.

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