VIP Anthropogenic Contamination | Very Important Paper

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Tramadol—A True Natural Product?**

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Abstract: *We have independently investigated the source of tramadol, a synthetic analgesic largely used for treating moderate to severe pain in humans, recently found in the roots of the Cameroonian medicinal plant, Nauclea latifolia. We found tramadol and its three major mammalian metabolites (O-desmethyltramadol, N-desmethyltramadol, and 4-hydroxycyclohexyltramadol) in the roots of N. latifolia and five other plant species, and also in soil and local water bodies only in the Far North region of Cameroon. The off-label administration of tramadol to cattle in this region leads to cross-contamination of the soil and water through feces and urine containing parent tramadol as well as tramadol metabolites produced in the animals. These compounds can then be absorbed by the plant roots and also leached into the local water supplies. The presence of tramadol in roots is, thus, due to an anthropogenic contamination with the synthetic compound.*

A recent study has shown that the synthetic drug tramadol ((±)-*cis*-2-[(dimethylamino)methyl]-1-(3-methoxyphenyl)cyclohexanol) is present as a "natural product" in the roots of the Cameroonian medicinal plant *Nauclea latifolia* (Rubiaceae).[1] Tramadol is a centrally acting opioid analgesic capable of weakly binding to μ-opioid receptors and inhibiting the reuptake of norepinephrine and serotonin.[2] Several synthetic drugs share a common core structure or even have an identical structure to compounds obtained from natural resources. For example, the main component of the ancient dye Tyrian Purple, which was initially isolated from molluscs, was later identified as dibromoindigo and synthesized.[3] The well-known anticoagulant and rodenticide warfarin is a synthetic derivative of the anticoagulant dicoumarol, which in turn is a derivative of the plant compound coumarin.[4,5] Similarly, the synthetic anticancer drug 5-fluorouracil (5-FU), which was initially designed and synthesized in 1957,[6] was much later isolated from the marine sponge *Phakellia fusca*.[7]

Notwithstanding the re-discovery of synthetic compounds from natural resources, the presence of tramadol in the roots of *N. latifolia* as reported by De Waard and co-workers[1] opens further questions on its biosynthetic pathway. Although the isolation, structural elucidation, and final chemical identity of tramadol was clearly demonstrated using high-resolution mass spectrometry as well as NMR and X-ray spectroscopic methods (which only provide evidence of the presence of tramadol and not its origin), we were particularly intrigued by the results of the isotope ratio analyses. The $\delta^{15}N/^{14}N$ and $\delta^{13}C/^{12}C$ ratios of the natural tramadol (natural 1 and natural 2) fell within the range of commercial samples used by the authors.[1] Therefore, the actual source of tramadol in the plant roots could not be conclusively confirmed by the isotope ratio measurements.

To independently verify the natural origin of tramadol, we conducted three different field campaigns (one in the dry season, one at the end of the dry season with very little rain, and one at the beginning of the rainy season) to obtain *N. latifolia* plants from two different locations in Cameroon with drastically different environmental conditions and lifestyles of the local inhabitants (see Tables S1–S3 and Figure S1 in the Supporting Information). One of our bioprospecting locations was Houdouvou (northwest of Maroua) in the Far North region of Cameroon[8] and the other was within the tropical rainforest area in Bafia in the central region of Cameroon in the south[9] (see the Supporting Information for a detailed description of the locations and sampling strategies). Our phytochemical analyses revealed some striking results:

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1) Although we could verify the findings of De Waard and co-workers[1] by detecting the presence of tramadol in the roots of *N. latifolia* prospected from the Far North region, the content of tramadol in our root samples ranged between 0.0000017 % (w/w) and 0.0001466 % (w/w; see Table S1 in the Supporting Information), compared to the reported high values of 0.4 % w/w[1] (which was also verified by us). Furthermore, we found either a complete absence (less than the limit of detection) or detectable amounts of tramadol in different root tissues of even the same plant (for example, Root1-3 M1 June; see Table S1 in the Supporting Information).

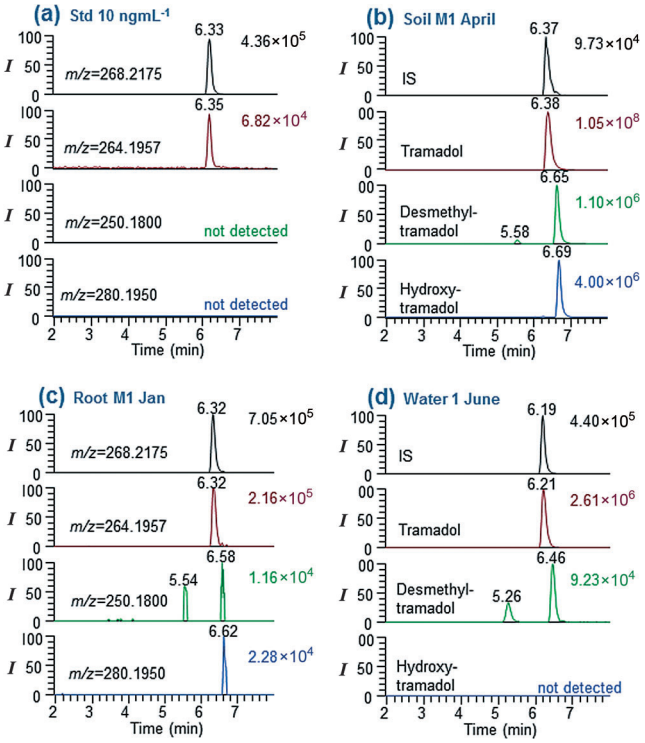

Figure 1. Selected mass traces (< 2 ppm) of the internal standard [^{13}C-D$_3$]tramadol, tramadol, *O*-desmethyltramadol (R_t = 5.5 min), *N*-desmethyltramadol (R_t = 6.6 min), as well as 4-hydroxycyclohexyltramadol. a) Reference compound tramadol, b) extract of soil (Soil M1 April), c) extract of *N. latifolia* root (Root M1 Jan), and d) surface water sample (Water 1 June).

2) None of the plants collected from the southern area (Bafia region) had any detectable amounts of tramadol.

Figure 1 shows the full-scan chromatograms of a tramadol reference standard (10 ng mL^{-1}), soil sample M1 April, root sample M1 Jan, and water sample Water 1 June (see Tables S1 and S3 in the Supporting Information). The retention time and accurate masses (*m*/*z* 264.1957) of the reference standard and tramadol present in the soil, root, and water samples were identical. Additionally, the presence of tramadol was confirmed in all samples by MS^2 experiments. A comparison of the HR-MS/MS spectra of tramadol and its labeled internal reference (^{13}C-D$_3$) clearly allowed identification of the compounds (see Figure S2 in the Supporting Information for a representative example).

Given the high variation in the tramadol content within the roots of *N. latifolia* plants prospected from the Far North region and occurrence of tramadol (in some cases) in the inner core of the root bark (similar to the original study[1]), we then hypothesized the possibility of endophytes associated with *N. latifolia* as being the original producers of tramadol. It is well known that endophytes, the microorganisms that harbor live tissues of plants in an asymptomatic and often mutualistic manner, are capable of producing a plethora of secondary metabolites with diverse and pharmaceutically relevant biological activities.[10] Particularly interesting are those plant-associated microorganisms harboring in specific ecological niches and able to produce metabolites mimetic to host plant compounds (for example, maytansine).[11,12] We, therefore, isolated both endophytic bacteria and fungi from the roots of fresh *N. latifolia* plants prospected from all the above locations. The endophytes were fermented in suitable media, extracted, and analyzed using HPLC coupled to a high-resolution mass spectrometer (see the Supporting Information). None of the isolated endophytic bacteria and fungi produced tramadol.

The contrast in the results obtained from plants sampled from the Far North and southern regions of Cameroon along with the negative results from endophytes prompted us to hypothesize that the variable amounts of tramadol detected in selected *N. latifolia* roots might in fact be an anthropogenic contamination by synthetic tramadol. We conducted a series of interviews with farmers and local inhabitants at our collection sites. As a result, the following information could be gathered:

1) An extensive off-label use of synthetic tramadol both by the farmers as well as their farm animals occurs only in the Far North region (Houdouvou). The farmers, who have to work throughout the day under the sun in extremely high temperatures, buy tramadol from the local market or from local street sellers (twelve pills cost less than one Euro). They consume around two to three pills, which is much higher than the daily recommended dosage, with their morning cup of tea. They report that this allows them to work all day without feeling tired.

2) Tramadol is further fed to cattle (but not to goats and sheep) as capsules when working them in their farms (as draft animals) so that the animals do not get tired quickly. As a consequence of the high day temperatures and the strong sun, the farm animals often choose the shade of trees to relax and also excrete their urine and feces.

3) Tramadol is also administered to horses prior to horse racing and is given only on the day of the competition. Generally around five capsules of tramadol (100 mg each) are mixed with flour and water to prepare a small cake, which is then fed to the horses before the race. Then, at the end of the day, the horses are "detoxified" by being fed milk.

4) In the southern region (Bafia), the use of tramadol is not known to farmers.

With this background information and after further consultation with the local farmers working at our bioprospecting locations, we then collected soil from the Far North

region, including from the vicinity of the *N. latifolia* plants, particularly from around the roots in the dry season and at the beginning of the rainy season (see Table S1 and Figure S1 in the Supporting Information). We also collected samples of surface, stream, and well water from different locations (see Table S3 and Figure S1 in the Supporting Information). In addition to the parent tramadol, three mammalian metabolites (*O*-desmethyltramadol, *N*-desmethyltramadol, and 4-hydroxycyclohexyltramadol; Figure 1 b) could be identified in

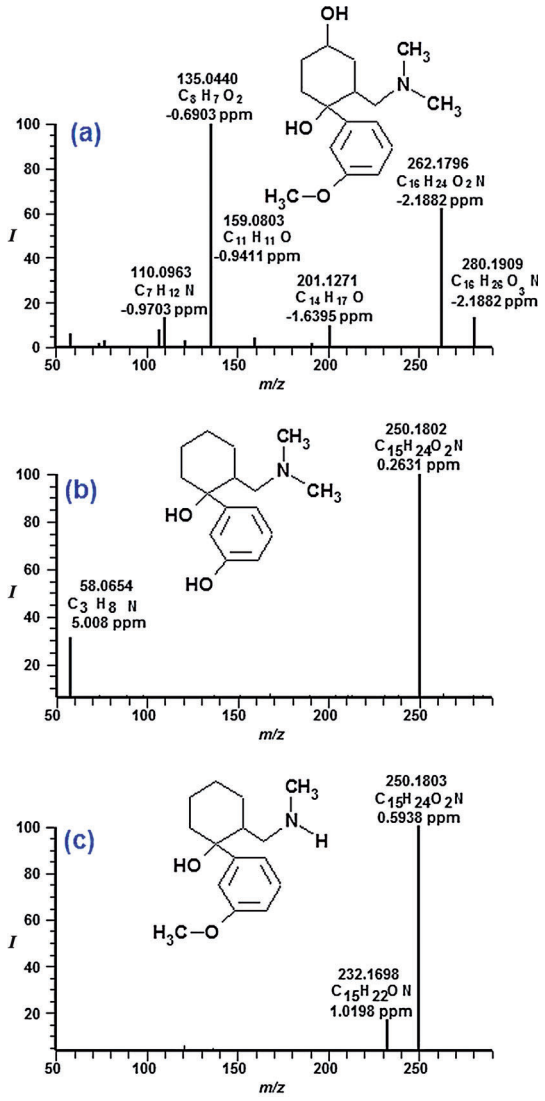

Figure 2. HR-MS/MS spectra of a) 4-hydroxycyclohexyltramadol, b) *O*-desmethyltramadol at $R_t = 5.5$ min with a prominent fragment at *m/z* 58, and c) *N*-desmethyltramadol at $R_t = 6.6$ min.

the soil samples. The compounds were attributed to the tramadol metabolites described by Wu et al. (2002)[13] through comparison of MS^2 data. The HR-MS/MS spectra of both the demethylated (*O*- and *N*-) and hydroxylated tramadol are given in Figure 2. The content of the metabolites relative to parent tramadol was estimated to be 1% to 10% from the intensities of the full scan traces (Figure 1). Interestingly, the water samples also contained tramadol (ranging from 36 to 553 $ng\,L^{-1}$) along with its demethylated metabolites (Figure 1 d). Although *N*- or *O*-demethylation can occur in plants, these metabolites would not be present in the soil or water.

Studies on the fate and metabolism of tramadol in vitro using human liver cells and in vivo in human volunteers demonstrated the persistence of the non-metabolized parent tramadol ($\geq 80\%$ in urine) along with its highly active metabolite *O*-desmethyltramadol.[13–15] Therefore, to confirm whether the presence of tramadol in *N. latifolia* roots is a result of cross-contamination caused by the absorption of tramadol and its metabolites excreted by cattle, we reinvestigated the plant roots to check for the tramadol metabolites we found in the soil. Very interestingly, the plant roots collected from the Far North also contained the three mammalian metabolites of tramadol in addition to the parent compound (Figure 1 c and Figure 2). Furthermore, our estimated content of mammalian tramadol metabolites compared to the parent non-metabolized tramadol is comparable to the reported values.[13] Finally, using the rationale that contamination of soil by tramadol might also lead to its accumulation in the roots of other plants, we sampled *Acacia polyacantha* (syn. *Senegalia polyacantha*), *Acacia sieberiana*, *Andira inermis*, and *Piliostigma reticulatum*, as well as *Ficus sycomorus* plants from the same area. Indeed, we could also detect various amounts of tramadol in their roots (see Table S2 and Figure S3 in the Supporting Information). This showed that tramadol and its metabolites present in the roots were not biosynthetic products of the plants or their endophytes, but a result of cross-contamination.

In conclusion, we have firstly shown that tramadol can be detected in the roots of *N. latifolia* and soil in the vicinity of the plants, as well as in water prospected only from the Far North region of Cameroon but not from the central region in the south. Secondly, endophytic microflora isolated from the tissues of these plants did not biosynthesize tramadol when cultured in vitro. Thirdly, several known human and animal metabolites were found in the roots, soil, and water along with the parent synthetic tramadol. Finally, several other plant species growing in the area also showed accumulation of tramadol in their roots. Our interviews revealed the off-label use of tramadol in humans and cattle in Houdouvou, which is supported by our experimental data. In this study, we have thus shown that tramadol and its metabolites detected in plant roots at particular regions is a consequence of anthropogenic cross-contamination. Similar incidents of cross-contamination of natural resources with synthetic compounds have been reported earlier. One prominent example is the deaths of vulture populations across the Indian subcontinent because of the off-label use of diclofenac in cows.[16,17]

The use of *N. latifolia* in traditional medicine (since it is known to have similar effects as tramadol) is now hindered because of cross-contamination with synthetic tramadol, which has severe side effects. There is documented evidence of adverse (and even fatal) health effects of tramadol from abuse[18] and intoxication, such as recurrent seizures,[19] multiple organ dysfunction syndromes,[20] and even death in humans.[21] The present off-label use of tramadol in certain regions of Cameroon, as exemplified in our study, may therefore pose a risk not only to humans and animals directly

dosed with tramadol but also through indirect exposure to drinking water because of the high-water solubility of tramadol. In fact, our current study also shows leaching of tramadol and contamination of water in the Far North region (see Figure 1 d and Tables S1–S3 in the Supporting Information). This is supported by soils sampled at the beginning of the rainy season (Soil M1 June) showing decreased tramadol values. Therefore, immediate measures should be taken to restrict the off-label use of tramadol in northern Cameroon. Our study highlights the problem of how anthropogenic contamination of natural resources such as plants by different synthetic compounds can lead to erroneous interpretations.

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