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Synthetic Origin of Tramadol in the Environment

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Dedicated to Professor Gerhard Spiteller

Abstract: *The presence of tramadol in roots of Sarcocephalus latifolius trees in Northern Cameroon was recently attributed to point contamination with the synthetic compound. The synthetic origin of tramadol in the environment has now been unambiguously confirmed. Tramadol samples isolated from tramadol pills bought at a street market in downtown Maroua and highly contaminated soil at Houdouvou were analyzed by high-precision ^{14}C measurements by accelerator mass spectrometry (^{14}C AMS): Tramadol from the pills did not contain any radiocarbon, thus indicating that it had been synthesized from ^{14}C-free petroleum-derived precursors. Crucially, tramadol isolated from the soil was also radiocarbon-free. As all biosynthetic plant compounds must contain radiocarbon levels close to that of the contemporary environment, these results thus confirm that tramadol isolated from the soil cannot be plant-derived. Analyses of S. latifolius seeds, in vitro grown plants, plants from different origins, and stable-isotope labeling experiments further confirmed that synthetic tramadol contaminates the environment.*

Synthetic tramadol was originally developed by Grünenthal GmbH in the 1960s as an easy-to-synthesize alternative to morphine.[1,2] Tramadol has an analgesic potency that is around 10% that of morphine, and inhibits the reuptake of norepinephrine and serotonin.[3,4] Furthermore, it has antidepressive and anxiolytic properties. The main pharmacologically active metabolite *O*-desmethyltramadol (*O*-DSMT) is significantly more potent than the parent compound. Off-label use of *O*-DSMT has been reported; it has been spiked into so-called "Krypton" herbal preparations of the tropical deciduous tree *Mitragyna speciosa*, which has led to several cases of lethality in Sweden since 2009,[5,6] aside from similar cases of abuse in Germany.[7] Tramadol was recently found to be present in roots of one *Sarcocephalus latifolius* (syn. *Nauclea latifolia*) tree.[8] However, it was later demonstrated that extensive off-label use of tramadol in humans and cattle in Northern Cameroon has led to its occurrence not only in *S. latifolius*, but also in roots of several other plant species, as well as to sporadic occurrence in soil and surface and ground water of the region.[9] As a result, the issue of the "natural" versus "synthetic" origin of tramadol in the environment has garnered significant attention.[10,11] More recently, an attempt was made to determine the origin of tramadol in only one root sample using position-specific ^{13}C NMR spectroscopy.[12] Thus far, however, the major open question has remained unanswered: Is the origin of tramadol in the environment natural or synthetic?

To fully answer this question, we employed three different strategies. First, we determined the radiocarbon content of 1) tramadol isolated from pills obtained from a street market in downtown Maroua, 2) tramadol isolated from highly contaminated soil (site M1)[9] in Houdouvou in Northern Cameroon, and 3) tramadol isolated from a *S. latifolius* root (site M5)[9] collected in Houdouvou using high-precision ^{14}C accelerator mass spectrometry (^{14}C AMS).[13–15] The rationale behind using the radiocarbon content to indicate a natural or synthetic origin for tramadol is the fact that natural organic molecules recently synthesized by living organisms must contain radiocarbon. Carbon-14 is a cosmogenic nuclide. The carbon atoms in natural compounds are either directly or indirectly derived from atmospheric carbon dioxide by photosynthesis; therefore, the radiocarbon content of natural molecules reflects the ^{14}C content of the contemporary atmosphere. Synthetic molecules are the products of synthetic organic chemistry. Synthetic compounds are frequently produced from petroleum-derived precursors. Petroleum compounds once were natural compounds derived from living plants; however, all traces of the carbon-14 atoms that they once contained have completely decayed. Therefore, all natural compounds must have the radiocarbon content of the contemporary atmosphere, whereas synthetic compounds will have radiocarbon contents that reflect the ^{14}C content of their precursors. Therefore, ^{14}C AMS clearly has advantages over the stable carbon and nitrogen isotope ratio measurements ($\delta^{13}C$ and $\delta^{15}N$) of tramadol isolated from different sources, which are not conducive to confirm their origins owing to the higher variability (see the Supporting Information, Table S1). Moreover, the recently published paper[12] on the position-specific distribution of ^{13}C in tramadol determined by ^{13}C NMR spectroscopy could not solve the above-mentioned question as to the natural or synthetic nature of tramadol. High-precision ^{14}C AMS revealed that tramadol samples isolated from the pills obtained from the street market and from soil are not of natural origin (Table 1). The pMC (percent modern carbon) value as well as the calculated biobased content confirmed that the tramadol traces in the

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Table 1: Determination of the biobased content by high-precision ^{14}C AMS.

	pMC [%]	Calculated biobased content [%]
Expected value if tramadol is of natural origin[a]	104[b]	–
Expected value if tramadol is of synthetic origin[a]	<3	–
Tramadol isolated from pills from Maroua street sellers	1.3 ± 0.1	1.2 ± 0.1
Tramadol isolated from the soil at site M1 (Houdouvou)	0.23 ± 0.1	0.2 ± 0.1
Tramadol isolated from *S. latifolius* roots growing at site M5 (Houdouvou) and the crude fraction remaining after isolation of tramadol	168–190[c]	159–180[c]

[a] According to the ASTM D-6866-12 definition of "biobased content".[15] [b] Taking into account incorporation of atomic-bomb-derived ^{14}C. [c] Contamination.

soil are clearly of synthetic origin. The tramadol content in almost all *S. latifolius* roots that we sampled was extremely low (or tramadol was absent) in contrast to the report by Boumendjel and co-workers (2013),[8] and therefore, our samples could not be used for ^{14}C AMS. However, one particular *S. latifolius* tree root that we found in Houdouvou (site M5)[9] was contaminated with 840 ng g^{-1} (dry weight, d.w.) tramadol, which was isolated for ^{14}C AMS measurements. Although this sample was mainly handled outside our isotope laboratory, unfortunately, it was found to be contaminated with one or more radiocarbon-rich synthetic compounds, leading to unexpectedly high pMC values ranging from 168 to 190 % (Table 1).

It is very difficult to find *S. latifolius* roots with enough tramadol that can be isolated for ^{14}C AMS (min. 200 µg). Therefore, to overcome the aforementioned problem and to determine whether tramadol is really plant-derived, we analyzed *S. latifolius* seeds obtained from The Royal Botanic Gardens, Kew, London (UK), which did not contain tramadol or its metabolites. After germinating the seeds and growing *S. latifolius* plants in vitro (see Figure S1), the roots, stem, and leaves were analyzed, and tramadol was found to be absent in all tissues. Furthermore, when *S. latifolius* was fed with synthetic tramadol in a hydroponic culture, tramadol was taken up by the plant roots in small quantities as expected, but none of the known or potential plant metabolites (see Table S2) could be detected. Moreover, *S. latifolius* was fed with L-phenyl-[D_5]-alanine in a hydroponic culture to confirm the theoretical biosynthetic pathway of tramadol that was recently proposed.[12,16] Although L-phenyl-[D_5]-alanine was taken up by *S. latifolius* and incorporated into its secondary metabolic pathway(s), as exemplified by the incorporation of deuterium into the biosynthetic intermediate chlorogenic acid (see Figures S2 and S3), neither labeled nor unlabeled tramadol could be found. Taken together, these results confirm that tramadol is not a plant secondary metabolite. Therefore, the reported "tramadol-like" activities of extracts from *S. latifolius* plants[17,18] are most likely to be either effects from tramadol-contaminated roots or from yet untested or unreported actual bioactive natural products of the plant.

Finally, to assess the persistence of synthetic tramadol in the environment and to determine the extent of tramadol abuse (see Figure S4), we conducted extensive field campaigns in Northern Cameroon in November 2014 at the end of the rainy season followed by a second one in February 2015 during the dry season and a third one in July/August 2015 after the rainy season had started again (see the Supporting Information for a detailed description of the locations and sampling strategies).

Sampling at Bénoué National Park: We analyzed *S. latifolius* roots, stems (bark), and leaves as well as fruits from five different locations across Bénoué National Park (BP1–BP5, see Figure S5 and Table S3) along with roots of other plants growing in the vicinity, such as *Pterocarpus latiflorus*, *Prosopis africana*, *Annona senegalensis*, *Acacia sieberiana* (syn. *Vachellia sieberiana*), *Combretum molle*, *Combretum micranthum*, and *Isoberlinia angolensis* (syn. *Isoberlinia tomentosa*). We also collected soil and surface water samples from locations within the park where *S. latifolius* trees were not found. Finally, we collected water from an 18.5 m deep public well near Guidjiba (site BP7, see Figure S5) to estimate the extent of tramadol contamination in the ground water. Interestingly, roots of only one *S. latifolius* tree (site BP5) contained very low amounts of tramadol at the end of the rainy season in 2014 (see Figure S5 and Table S3). Remarkably, roots of other plants growing in the vicinity of the *S. latifolius* tree contaminated with tramadol, such as *C. micranthum*, also contained tramadol in similar amounts, thereby confirming the point contamination with tramadol in only these particular regions of the park. However, during our second campaign during the dry season in February 2015, tramadol could not be detected in the exact same plants, confirming the leaching of the highly water-soluble tramadol because of the heavy rain in November 2014. Large amounts of tramadol could be detected in the public-well water samples collected at BP7 during our first campaign (1610 ng L^{-1}) but also during our second campaign (325 ng L^{-1}), along with the highly active metabolite *O*-DSMT, as well as *N*-desmethyltramadol (*N*-DSMT), and 4-hydroxycyclohexyltramadol (4-HCHT; see Table S3).

Sampling at a village near Bénoué National Park: Widespread contamination of the public wells with tramadol could also be observed at our second sampling site at a nearby village. Although we could not detect tramadol or its metabolites in *S. latifolius* or other plants or in the soil, three public wells (V5 to V7, ca. 15 m deep) were found to be contaminated with tramadol (up to 27 ng L^{-1}; see Table S3), indicating tramadol contamination of the nearby environment. The highly variable occurrence of tramadol in this area, ranging from its complete absence in plants and soil to high amounts in water, exemplifies the point contamination with tramadol.

Sampling at Houdouvou: We re-sampled *S. latifolius* roots, soil, and water at our first campaign site in Houdouvou, which is the location where anthropogenic point contaminations with tramadol and its metabolites was originally found.[9] Three striking observations could be made upon analyzing the samples: First, roots of an *S. latifolius* tree at site M5, where no tramadol had been found in June 2014,[9] contained very large amounts of tramadol when sampled in November 2014 (840 $ng\,g^{-1}$). Second, soil collected from the vicinity of an *S. latifolius* tree at site M1, which contained 1470 $ng\,g^{-1}$ tramadol in April 2014 and only 21 $ng\,g^{-1}$ in June 2014 after the first showers,[9] showed an extremely high amount of tramadol in soil clumps (less than 1 kg) when sampled again in November 2014 (20800 $ng\,g^{-1}$). The high amounts and variability of the tramadol content in the soil at site M1 represent a point-source contamination "hot spot", and may be similar to the contamination of a root sample reported to contain extremely high amounts of tramadol.[8] Third, highly variable amounts of tramadol could also be detected in surface water collected throughout the location (see Table S3).

Finally, we also collected and analyzed *S. latifolius* roots from Ghana (Asakraka Kwahu) and *S. latifolius* roots procured from a traditional healer in downtown Maroua, none of which contained tramadol or its metabolites.

Sampling in downtown Maroua: To estimate the effect of off-label tramadol use in the urban areas of Cameroon, we performed extensive sampling in downtown Maroua (Figure 1 and Figure S6). Strikingly, water samples across the entire city of Maroua were contaminated with tramadol and its metabolites (see Table S3). This included water from the vicinity of the three rivers flowing through the city, namely Mayo Ziling, Mayo Kalliao, and Mayo Tsanaga. Interestingly, surface water samples collected by digging around 50 cm into the river bed at "Quatier pont", 500 m from the Green Bridge over the Mayo Kalliao river, were found to contain extremely high amounts of tramadol (49400 $ng\,L^{-1}$) along with its metabolites. These extremely high values can be explained by the fact that during the dry season, evaporation of surface water occurs throughout Maroua, which increases the tramadol concentration in the remaining water. No trees could be found in the contaminated river bed, which corroborates that the extensive contamination of water with tramadol cannot be due to leaching from trees. Moreover, we re-sampled the highly contaminated water sources in July/August 2015, which confirmed their systematic contamination with tramadol even after heavy rainfall (sites WS7–9, Table S3). During our campaign, it was further observed that during the dry season, river beds are occupied by homeless people, who also use the tramadol-contaminated water for drinking and cooking. The river beds are also used as roads by inhabitants and their cattle, horses, sheep, and goats (see Figure S7).

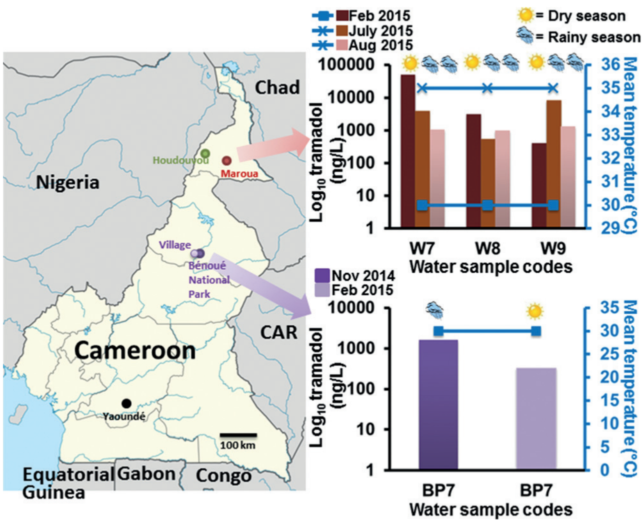

Figure 1. Sampling locations in Northern Cameroon for the collection of plant, soil, and water samples. Data for selected sites within downtown Maroua and Bénoué National Park that were extensively contaminated with tramadol are given on the right along with the climatic conditions during sampling (see also Table S3). The map of Cameroon was adapted and modified under the Creative Commons Attribution-Share Alike 3.0 Unported License (CC BY-SA 3.0).

In conclusion, we have unequivocally confirmed the presence of synthetic tramadol in the environment in Northern Cameroon. Tramadol addiction is known to cause adverse, and in some cases lethal, health effects, such as recurring seizures, intoxication, multiple organ dysfunction syndromes, and human fatalities.[19–22] Therefore, urgent measures must be taken to restrict the off-label use of tramadol, and further research is needed to assess the long-term side effects of tramadol in humans, animals, and the environment. The present study has implications not only for Cameroon but also for other countries where high consumption and improper disposal of pharmaceuticals have led to wide-spread contamination of the environment. Some well-known examples include the near-extinction of Asian vultures following cattle-mediated exposure to the anti-inflammatory drug diclofenac,[23–25] an increased cross-resistance of human pathogens to fluoroquinolone antibiotics, such as ciprofloxacin, owing to the use of enrofloxacin in veterinary medicine,[26] and the ecotoxicological problem of "feminized fish" in the aquatic ecosystem, which is due to contamination with synthetic 17α-ethinylestradiol and 17β-estradiol.[27,28] The World Drug Report 2015 of the United Nations Office on Drugs and Crime (UNODC)[29] as well as the EU Drug Policy of 2015[30] attest to the urgency of addressing the problem of anthropogenic contamination of the environment with pharmaceuticals around the world.

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