A GC-MS Protocol for the Identification of Polycyclic Aromatic Alkaloids from Annonaceae

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Abstract: The Annonaceae are an old family of flowering plants belonging to the order Magnoliales, distributed mainly in tropical regions. Numerous Annonaceae species find ethnobotanical use for curing a broad range of diseases, among them cancer and infections by diverse pathogens. Hence, bioactive natural products from Annonaceae have received considerable interest in drug development. Beyond cytotoxic acetogenins, unique aporphine-derived polycyclic aromatic alkaloids are characteristic constituents of Annonaceae. Among them are unique tri- and tetracyclic aromatic alkaloids like azafluorenones, diazafluoranthenes, azaanthracenes, and azaoxoaporphines. The complex substitution pattern of these alkaloids represents a major challenge in structure elucidation of isolated natural products. Based on a broad spectrum of alkaloids available from our previous work, we present a GC-MS protocol for the identification of over 20 polycyclic aromatic alkaloids from Annonaceae. This collection of data will contribute to the future identification of the metabolite patterns of extracts from Annonaceae as an important source of novel bioactive secondary metabolites.

Keywords: alkaloids; Annonaceae; azafluorenones; diazafluoranthenes; oxoaporphines; azaoxoaporphines; gas chromatography; mass spectrometry

1. Introduction

The Annonaceae are an old family of flowering plants, mainly trees and shrubs, from the order Magnoliales. Found mainly in tropical regions, they have considerable economic significance as sources of edible fruits (custard apple, cherimoya, soursop) and essential oils (e.g., ylang-ylang oil from the flowers of *Cananga odorata* used in perfumes) among local populations. Furthermore, extracts (mainly from barks and roots) have found ethnobotanical use in the curing of a broad range of diseases, among them infections by diverse pathogens and tumors [1].

Over decades, unique bioactive secondary metabolites have been isolated from Annonaceae. Acetogenins, a class of $C_{35}/C_{37}$ metabolites derived from long chain ($C_{32}/C_{34}$) fatty acids in the polyketide pathway, represent a very prominent chemotype among Annonaceae constituents. Exhibiting impressive antitumor activity, they, nonetheless, never reached the market due to severe toxicity, especially neurotoxicity [2]. A second prominent group of natural products from Annonaceae which received considerable interest in drug development are polycyclic aromatic alkaloids, most of which are biosynthetically derived from aporphinoid precursors. Oxoaporphines, well-known tetracyclic metabolites from the benzylisoquinoline pathway, are speculated [3,4] to undergo further transformations—including ring cleavage—during late-stage metabolism, as depicted in Figure 1. These proposed biotransformations produce unique tri- and tetracyclic aromatic alkaloids like azafluorenones, diazafluoranthenes, and azaoxoaporphines. Typical of the biosynthetically well-studied tyrosine-derived aporphinoid alkaloids, these natural products bear characteristic hydroxylation and methoxylation patterns.
Figure 1. Postulated biosynthesis of azaaoxaporphines (e.g., sampangine, (5)), diazafluoranthenes (e.g., eupolauridine, (6)), azaanthraquinones (e.g., cleistopholine, (9)), and azafluorenones (e.g., onychine, (10)) starting from aporphinoid precursors.

In its infancy, structural analysis of natural products was primarily reliant on chemical degradation and derivatization reactions of the target molecules, analytical methods which have since been largely replaced by modern alternatives including multidimensional NMR (nuclear magnetic resonance), UV (ultraviolet), IR (infrared), CD (circular dichroism), X-ray crystallography, and MS (mass spectrometry) data, or proof of postulated structures by unambiguous total synthesis [5]. Naturally, structure elucidation is rarely based on a single analytical method but, rather, on mutually supportive data gathered from multiple sources. However, even despite these advancements, structural misassignments of alkaloids from this pathway occurred in the past [6–9], and identification of the precise substitution patterns of newly isolated Annonaceae alkaloids remains challenging. For example, the structures of annonaceous 4-aza-1-methylfluorenones onychine (10), first isolated from Onychopetalum amazonicum, and 6-methoxyonychine (13), first isolated from Guatteria dielsiana, were misreported as 1-aza-4-methylfluorenones on the basis of IR, UV, EIMS, HRMS, and $^1$H NMR data [10,11]. These structure misassignments were retained in the literature across several years in the 1980s until revision by total synthesis of the proposed structures [6]. We also showed that the postulated [11] and later revised [6] structure for 4-azafluorinde dielsine isolated from Guatteria dielsiana as 4-methyl-1H-indeno[1,2-b]pyridine-2,5-dione remains erroneous through total synthesis in previous works of ours [9]. Phytochemical analysis of newly investigated compounds from Annonaceae has to, therefore, rely on precise knowledge on previously isolated natural products with confirmed structures. Based on a representative library of synthesized alkaloids from decades of our own research in this field, we developed a convenient GC-MS (gas chromatography–mass spectrometry) protocol for the identification of polycyclic aromatic alkaloids from Annonaceae. Further, we included a number of synthetic congeners which have not (or not yet) been identified as natural products. In total, we present chromatographic and mass spectrometric data for 25 compounds of interest.

2. Results and Discussion

2.1. Source of Analytes

Based on long-term work on the total synthesis of alkaloids from Annonaceae, we compiled an in-house substance library of 25 relevant compounds (Table 1 and Figure 3), covering all relevant chemotypes of aporphine-derived aromatic alkaloids typical of (some of them even unique to) Annonaceae. It includes representatives of well-known, broadly
distributed alkaloid classes (1-benzylisoquinolines, oxoaporphines) as well as aporphine-
derived chemotypes (azaoxoaporphine, diazafluoranthene, azaanthraquinone, azafluoro-
renone) characteristic of Annonaceae. Our efforts also furnished the unique lactone alkaloid
polynemoraline C (24). Interestingly, this alkaloid was found to be accompanied by several
aporphinoid alkaloids in Polyalthia nemoralis (Annonaceae) [12]. With the pyrimidyl-β-
carboline annomontine (25) [13] we also included an Annonaceae alkaloid which arises
from a distinct biosynthetic pathway (tryptophan-derived). Representative citations for
the biological sources of these alkaloids are given in Table 1; information about the total
syntheses is given in Section 3.1 Materials and Reagents.

The alkaloid collection was supplemented by a couple of synthetic azafluorenones
which have not yet been identified as natural products. (Note: 5,6-Dimethoxyonychine (20)
and 5,8-dimethoxyonychine (22) were mentioned as “alkaloids” by Zhang [7]. In fact, these
compounds were only synthesized in order to compare the spectroscopic data with those
of alkaloids isolated from Oxandra xylopoide).

Figure 2. Total ion chromatogram of the compounds 3–25; 3 O,O-dimethylannocherine A,
4 lysicamine, 5 sampangine, 6 eupolauridine, 7 eupolauridine mono-N-oxide, 8 eupolauridine
di-N-oxide, 9 cleistopholine, 10 onychine, 11 ursuline, 12 isoursuline, 13 6-methoxyonychine,
14 darienine, 15 polyfothine, 16 5,6,7,8-tetramethoxyonychine, 17 7-hydroxy-5,8-dimethoxyonychine,
18 7-methoxyonychine, 19 muniranine, 20 5,6-dimethoxyonychine, 21 3-methoxyonychine, 22 5,8-
dimethoxyonychine, 23 5,7,8-trimethoxyonychine, 24 polynemoraline C, 25 annomontine; * impurities.
Due to the individual volatility and thermal stability of the compounds, different concentrations were
used in order to obtain a chromatogram visualizing the chromatographic behavior of all analytes
(3 100 µg/mL, 4–6 20 µg/mL, 7, 8 50 µg/mL, 9–25 and 15 20 µg/mL.).
Table 1. Alkaloids (and synthetic analogues) analyzed in this investigation, including information on biological sources, chemical structure, MS data, and chromatographic properties (see Figure 2). * Not described as a natural product; ** not isolated from Annonaceae, but from Nelumboaceae; ¹ CAS = Chemical Abstract Services; ² RRT = relative retention time; ³ temperature-programmed Kováts retention index.

| Code | Trivial Name [Biological Source] | CAS ¹ Number | Chemical Formula | M [g/mol] | Characteristic Ions [m/z] (Base Peak in Bold) | RRT ² (Fluorene) | Kováts Index ³ |
|------|---------------------------------|--------------|-----------------|-----------|-----------------------------------------------|-----------------|----------------|
| 1    | Annocherine A [14]              | 344928-12-7  | C₁₅H₂₁NO₄       | 297.10    | (I) 280, 265, 220                               | 1.818           | 2925           |
|      |                                 |              |                 |           | (II) 279, 264, 236                             | 1.985           | 3295           |
| 2    | Annocherine B [14]              | 344928-13-8  | C₁₃H₁₇NO₄       | 311.12    | (I) 280, 265, 220                               | 1.818           | 2925           |
|      |                                 |              |                 |           | (II) 279, 264, 236                             | 1.985           | 3295           |
| 3    | O,O-Dimethylannocherine A ** [15]| 1268489-61-7 | C₁₃H₁₇NO₄       | 325.13    | (I) 322, 308, 292                               | 1.872           | 3045           |
|      |                                 |              |                 |           | (II) 308, 294, 278                             | 1.750           | 2784           |
|      |                                 |              |                 |           | (III) 307, 292, 248                            | 1.937           | 3190           |
| 4    | Lysicamine [16]                 | 15444-20-9   | C₁₅H₂₀NO₃       | 291.09    | 291, 248, 177                                  | 1.964           | 3258           |
| 5    | Sampangine [17]                 | 116664-93-8  | C₁₅H₂₀NO₂       | 232.06    | 232, 204, 151                                  | 1.663           | 2614           |
| 6    | Eupolaridine [18]               | 58786-39-3   | C₁₄H₁₆NO₂       | 204.06    | 204, 177, 150                                  | 1.346           | 2074           |
| 7    | Eupolaridine mono-N-oxide [19]  | 96889-95-1   | C₁₅H₂₂O₂        | 220.06    | 220, 204, 165                                  | 1.630           | 2552           |
| 8    | Eupolaridine di-N-oxide [19]    | 96889-96-2   | C₁₅H₂₂O₄        | 236.06    | 236, 220, 204                                  | 1.885           | 3072           |
| 9    | Cleistopholine [19]             | 96889-94-0   | C₁₅H₂₀NO₂       | 223.06    | 223, 195, 167                                  | 1.467           | 2267           |
| 10   | Onychine [10]                   | 58787-04-5   | C₁₃H₁₄NO        | 195.07    | 195, 166, 139                                  | 1.171           | 1823           |
| 11   | Ursuline [20]                   | 111316-34-8  | C₁₃H₂₂O₃        | 241.07    | 241, 223, 183                                  | 1.430           | 2204           |
| 12   | Isoursuline [21]                | 112368-57-7  | C₁₃H₂₂O₃        | 241.07    | 241, 212, 198                                  | 1.481           | 2290           |
| 13   | 6-Methoxyonychicine [11]        | 105418-67-5  | C₁₃H₁₂NO₂       | 225.08    | 225, 182, 154                                  | 1.386           | 2135           |
| 14   | Darienine [23]                  | 111316-27-9  | C₁₃H₂₁NO₂       | 271.08    | 271, 256, 225                                  | 1.546           | 2400           |
| 15   | Polyfthamine [22]               | 122908-91-2  | C₁₃H₂₂O₃        | 255.09    | 255, 212, 169                                  | 1.529           | 2371           |
| 16   | 5,6,7,8-Tetramethoxychicine [23]| N/A          | C₁₃H₂₂O₃        | 315.11    | 315, 300, 239                                  | 1.621           | 2536           |
| 17   | 7-Hydroxy-5,8-dimethoxychicine [24]| N/A      | C₁₃H₂₂O₄        | 271.08    | 271, 242, 172                                  | 1.566           | 2436           |
| 18   | 7-Methoxyonychicine [25]        | 117719-70-7  | C₁₃H₂₁NO₂       | 225.08    | 225, 210, 154                                  | 1.369           | 2108           |
| 19   | Munitarine [26]                 | N/A          | C₁₃H₂₁NO₂       | 301.10    | 301, 283, 200                                  | 1.653           | 2596           |
| 20   | 5,6-Dimethoxyonychicine *       | 112368-58-8  | C₁₃H₂₂O₃        | 255.09    | 255, 254, 226                                  | 1.494           | 2312           |
| 21   | 3-Methoxyonychicine *           | 145013-64-5  | C₁₃H₂₁NO₂       | 225.08    | 225, 224, 196                                  | 1.290           | 1989           |
| 22   | 5,8-Dimethoxyonychicine *       | 112368-60-2  | C₁₃H₂₂O₄        | 255.09    | 255, 254, 226                                  | 1.516           | 2349           |
| 23   | 5,7,8-Trimethoxyonychicine *    | N/A          | C₁₃H₂₂O₄        | 285.10    | 285, 270, 256                                  | 1.612           | 2519           |
| 24   | Polyemoraline C [12]            | 1129491-73-1 | C₁₅H₁₉NO₆     | 271.09    | 271, 228, 185                                  | 1.617           | 2528           |
| 25   | Annomontine [13]                | 82504-00-5   | C₁₇H₁₁N₃        | 261.10    | 261, 245, 220                                  | 1.816           | 2920           |
Figure 2. Structures of the alkaloids (and synthetic analogues) analyzed in this investigation.

Figure 3. Structures of the alkaloids (and synthetic analogues) analyzed in this investigation.
2.2. GC-MS Analysis

A 5% phenyl polymethylsiloxane fused-silica capillary column was used as the stationary phase due to its well-known high separation efficiency, robustness, and its broad use in GC-MS analysis. To determine the retention behavior of each substance, we used the RRT in relation to the internal standard (IS) fluorene and the temperature-programmed Kováts retention index (or linear retention index, LRI) (Equation (1)).

\[
I = 100 \times \left( \frac{t_{RI} - t_{RZ}}{t_{R(z+1)} - t_{RZ}} + z \right)
\]

(1)

\[\begin{align*}
I &= \text{Kováts retention index} \\
I &= t_{RI} = \text{total retention time of the substance} \\
t_{RZ} &= \text{total retention time of the n-alkane} \\
z &= \text{number of the carbon atoms of the n-alkane} \\
t_{R(z+1)} &= \text{total retention time of the n-alkane} + 1 \text{ carbon atom}
\end{align*}\]

Equation 1. Determination of Kováts retention indices in case of temperature-programmed gas chromatography according to IUPAC (International Union of Pure and Applied Chemistry) [27].

As a proof of concept, the calculated indices of fluorene (IS), lysicamine (4), and onychine (10) were compared to published indices in the NIST (National Institute of Standards and Technology) Database [28]. We determined for IS a LRI of 1599 iu. The NIST Database estimated a retention index of 1494 iu and determined an average index of 1574 iu for 5% phenyl polymethylsiloxane capillary columns, which is close to our determined index. For lysicamine (4) and onychine (10), the NIST Database only estimated Kováts retention indices of 2465 iu and 1772 iu; our experimentally determined linear retention indices were 3258 iu and 1823 iu. For all other substances, no Kováts retention indices were available in the NIST Database. The linear retention indices of 25 polycyclic aromatic alkaloids from Annonaceae and related compounds are provided in Table 1. Additional chromatographic parameters of the analytes are listed in Table S1 (Supplementary Material).

Using a moderate heating rate (10 °C/min), it was possible to separate nearly all analytes of interest. The separation of the alkaloids 5,6,7,8-tetramethoxyonychine (16) and polynemoraline C (24) (Figure 3) was impossible based on their retention behavior. Both substances showed a similar RRT of 1.621 and 1.617 and Kováts retention indices 2536 iu and 2528 iu, respectively (Figure 2). However, they differed significantly in their mass spectra, which made identification easy (base peaks m/z 315 and m/z 271, respectively). The use of stationary phases with a modified selectivity (e.g., mid-polar columns with 50% phenyl 50% methylpolysiloxane) might allow separation of co-eluting compounds like (16) and (24).

Similarly, the differentiation of the benzylisouquinolines annocherine A (1) and annocherine B (2) based on their respective retention behavior and their mass spectra failed (see Supplementary Material). Due to their poor volatility, the peak intensities were low, even at an inlet temperature of 300 °C. The substances were not detectable at lower inlet temperatures, and the use of 300 °C inlet temperature led to degradation into 11/11I and 21/21II during injection (see chromatograms in the Supplementary Material). Fragments 11/21I showed the same RRT of 1.818, and a base peak at m/z 280. Even 11II/21II showed the same RRT of 1.985 and a base peak at m/z 279. Differentiation on the basis of the mass spectra also failed. Both substances showed similar MS spectra (EI, 70 eV). The molecular ion peak [M+] of 1 (m/z 279) and (2) (m/z 311) could not be observed in any mass spectrum of 11/11II and 21/21II. The volatility of the third benzylisouquinoline, O,O-dimethylannocherine A (3) was higher compared to 1 and 2. However, alkaloid 3 partially degraded into 31I and 31II during injection and the [M+] at m/z 325 could not be observed. Thus, the investigated 1-benzylisouquinolines proved challenging for GC-MS analysis. The most likely cause is the presence of the uncommon hydroxy/methoxy substituent at the benzylc sp² carbon, which leads to fragmentation under the thermal stress conditions in the injector block of the gas chromatograph. We suppose that the driving force for these thermal fragmentations is the
formation of highly conjugated systems like enamines and \( p \)-quinone methides (Figure S1, Supplementary Material). Since \( p \)-quinone methide formation is not plausible with the \( p \)-methoxybenzyl compound 3, this alkaloid exhibits higher thermal stability compared to the \( p \)-hydroxybenzyl alkaloids 1 and 2.

Fortunately, eupolauridine mono-\( N \)-oxide (7) and eupolauridine di-\( N \)-oxide (8) were degraded in a plausible manner during injection. The chromatogram of the mono-\( N \)-oxide (7) showed a peak of the mono-\( N \)-oxide (15%) and a peak of deoxygenation product eupolauridine (6, 85%), whereas eupolauridine di-\( N \)-oxide (8, 5%) was degraded into the mono-\( N \)-oxide (7, 30%) and eupolauridine (6, 65%; see chromatograms in the Supplementary Material). The base peaks of both \( N \)-oxides were \( m/z \) 204, but \([M^+\] peaks were present as well for both eupolauridine mono-\( N \)-oxide (7, \( m/z \) 220) and eupolauridine di-\( N \)-oxide (8, \( m/z \) 236). The differentiation of these alkaloids is possible, but thorough examination of the chromatograms is required to avoid misidentification of the degradation product as the analyte.

In summary, polycyclic aromatic alkaloids from Annonaceae were clearly identifiable except for the aforementioned benzylishoquinolines (1–3). Out of 25 substances, 14 showed a molecular ion peak \([M^+\] identical to the base peak (4–6, 9–11, 13, 15–18, 23–25), which facilitates identification.

### 3. Materials and Methods

#### 3.1. Materials and Reagents

The analytes (see Table 1 and Figure 3) were taken from our in-house substance library, compiled from previous and very recent work on natural product total synthesis, including a novel radical cyclization approach for azafluorenone [29]. All other syntheses are described in detail in our previous publications:

Azafluorenone alkaloids onychine (10) [30], ursuline (11) [29], isoursuline (=oxylop-\( \alpha \)-ine; 12) [29], 6-methoxyonychine (13) [31,32], darienine (14) [29], polyfothine (15) [29], 5,6,7,8-tetramethoxyonychine (16) [29], 7-hydroxy-5,8-dimethoxyonychine (17) [29], 7-\( \alpha \)-methoxyonychine (18) [29], and muniranine (19) [29], as well as synthetic analogues 5,6-dimethoxyonychine (20) [29], 3-methoxyonychine (21) [9], 5,8-dimethoxyonychine (22) [29], and 5,7,8-trimethoxyonychine (23) [29].

Sampangine (5) [33,34] is the most prominent member of azaan-\( \alpha \)-oaporphine alkaloids, eupolauridine (6) [30] as well as its mono- (7) and di-\( N \)-oxide (8) [35] are representatives of the diazafluoranthene alkaloids, and cleistophone (9) [33] was the first azaan-thraquinone alkaloid isolated from Annonaceae. As representatives of the postulated biosynthetic precursors of these unique aromatic alkaloids, we included the oxaaporphine lysicamine (4) [36] and three 1-benzylishoquinoline alkaloids: annoncherine A (1), annoncherine B (2), and \( O,O \)-dimethylannoncherine A (=annoncherine D; 3) [36], the latter of which is an alkaloid that, to date, has only been isolated from Nelumbonaceae plants.

Further, we included the annonaceous lactone alkaloid polymoraleine (24) [29] and the pyrimidyl-\( \beta \)-carboline alkaloid annonmontine (25) [37,38] in the investigation.

For the determination of the temperature-programmed Kov\( \acute{\text{a}} \)ts retention indices, alkane standards C7–C30 saturated (1000 mg/mL in \( n \)-hexane), alkane standard C10–C40 (all even, 50 mg/mL in \( n \)-heptane), as well as acetonirole of HPLC (high performance liquid chromatography)-grade were obtained from Sigma Aldrich (Schnelldorf, Germany). The internal standard (IS) fluorene was purchased from HPC Standards GmbH (Cunnersdorf, Germany).

Stock solutions of each compound (1 mg/mL) were prepared in acetonitrile and stored at 4 °C. Working solutions (in acetonirole) for each experiment were prepared in following concentrations: compounds 4–6 and 9–25, and IS at 20 µg/mL; compounds 7 and 8 at 50 µg/mL; compounds 1–3 100 µg/mL.
3.2. Instruments and Equipment

Gas chromatography (GC) was performed with a 7820A gas chromatograph from Agilent Technologies (Santa Clara, CA, USA). The G4514A autosampler and the G4513A injector were from Agilent Technologies (Santa Clara, CA, USA). Instrument control and data analysis were carried out with a Masshunter 8.0 from Agilent. An HP-5-ms capillary column of 30 m length, 0.25 mm i.d., and 0.25 µm film thickness was used at a constant flow rate of 1.2 mL/min. Helium 99.999% from Air Liquide (Düsseldorf, Germany) was used as carrier gas. The inlet temperature was kept at 300°C and the injection volume was 2 µL with splitless time 0.5 min. The initial column temperature was 50°C and held for 1 min. Then, temperature was ramped up to 320°C (10°C/min) and held for 2 min. The total run time was 30 min. MS source temperature was 230°C and quadrupole temperature was 150°C. The MS was operated with electron ionization (EI) at 70 eV in scan mode (m/z 40–600) with a solvent delay of 7.0 min.

4. Conclusions

The herein described gas-chromatographic system is able to separate annonaceous alkaloids from all relevant chemotypes (see Figure 1) efficiently, and in combination with MS detection represents a powerful and fast tool for the unambiguous identification of known alkaloids. By collecting chromatographic and mass spectroscopic data for an unprecedentedly large library on annonaceous alkaloids, we present here a valuable database for other scientists in this field. This method is only limited by the thermal instability of single chemotypes (benzylisoquinolines with benzylic oxygenation, N-oxides), where a very careful analysis of chromatographic and MS data is afforded. Since reports on the identification of novel alkaloids from Annonaceae continue to be published, our work should further contribute to the fast and secure analysis of alkaloid patterns and alleviate the structure elucidation of hitherto unknown alkaloids. Furthermore, the inclusion of some synthetic analogues (especially azafluorenones) which have not (yet) been identified as natural products might contribute to structure elucidations pertaining to the concept of “natural product anticipation through synthesis” [39].

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/molecules27238217/s1, Figure S1: Degradation; Table S1: Additional chromatographic parameters of the analytes; File S1: Chromatograms of the problematic compounds 1, 2, 3, 7, and 8; File S2: Substance data sheets for the single analytes (including retention data and MS spectra), and a scheme showing proposed fragmentation patterns of 1-benzylisoquinoline alkaloids 1–3.

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References

1. Lúcio, A.S.S.C.; Almeida, J.R.G.D.S.; Da-Cunha, E.V.L.; Tavares, J.F.; Filho, J.M.B. Chapter Five—Alkaloids of the Annonaceae: Occurrence and a Compilation of Their Biological Activities. In The Alkaloids: Chemistry and Biology; Knölker, H.-J., Ed.; Academic Press: Cambridge, MA, USA, 2015; Volume 74, pp. 233–409. [CrossRef]

2. Neske, A.; Hidalgo, J.R.; Cabedo, N.; Cortes, D. Acetogenins from Annonaceae family. Their potential biological applications. Phytochemistry 2020, 174, 112332. [CrossRef][PubMed]

3. Taylor, W. Constituents of Eupomatia species. IX. NMR evidence for the structure of eupolauramine and hydroxyeupolauramine. Aust. J. Chem. 1984, 37, 1095–1104. [CrossRef]

4. Tadić, D.; Cassels, B.K.; Leboeuf, M.; Cavé, A. Alkaloids of the Annonaceae. Part 69. Kinabaline and the aporphinoid biogenesis of azaanthraceane and azafluorene alkaloids. Phytochemistry 1987, 26, 537–541. [CrossRef]

5. Cavé, A.; Tadić, D.; Cassels, B.K. Spectral Properties of Ring-C-Oxygenated 4-Azafluorenones and 4-Azafluorenolones. The structures of Natural Onychine Derivatives. Heterocycles 1987, 26, 1551–1552. [CrossRef]

6. Tadić, D.; Cassels, B.K.; Cavé, A.; Goulart, M.O.; De Oliveira, A.B. Revised structures of the azafluorenone alkaloids from guatteria dielsiana. Phytochemistry 1987, 26, 1511–1523. [CrossRef]

7. Zhang, J.; El-Shabrawy, A.-R.O.; El-Shanawany, M.A.; Schiff, P.L.S., Jr.; Slatkin, D.J. New Azafluorene Alkaloids from Oxandra cf. major and biogenetic considerations. Phytochemistry 1987, 26, 537–541. [CrossRef]

8. Irie, H.; Koyama, J.; Sugita, T.; Suzuta, Y. Synthesis of an Alkaloid Onychine and Related Compounds: Revised Structure of Onychine. Heterocycles 1979, 12, 1017–1019. [CrossRef]

9. Bracher, F. Polycyclic aromatic alkaloids. VII. Studies on the structure of dielsine. Arch. Pharm. 1992, 325, 645–648. [CrossRef]

10. De Almeida, M.L.; Braz-F, R.; Von Bülow, V.; Gottlieb, O.R.; Maia, J.S. Onychine, an alkaloid from Onychopetalum amazonicum. Phytochemistry 1979, 15, 1186–1187. [CrossRef]

11. Goulart, M.O.; Santana, A.E.G.; De Oliveira, A.B.; De Oliveira, G.G.; Maia, J.G.S. Azafluorenones and azaanthraquinonone from Guatteria dielsiana. Phytochemistry 1986, 25, 1691–1695. [CrossRef]

12. Lu, Z.-M.; Zhang, Q.-J.; Chen, R.-Y.; Yu, D.-Q. Four new alkaloids from Polyalthia nemoralis (Annonaceae). J. Nat. Prod. 1987, 50, 800–806. [CrossRef]

13. Leboeuf, M.; Andre, C.; Forgacs, P.; Provost, J.; Chiaroni, A.; Riche, C. Alkaloids of the Annonaceae. Part 33. Annomontine and methoxyannomontine, two new pyrimidine-beta-carboline-type alkaloids from Annona montana. J. Chem. Soc. Perkin Trans. 1982, 1, 1205–1208. [CrossRef]

14. Chen, C.-Y.; Chang, F.-R.; Pan, W.-B.; Wu, Y.-C. Four alkaloids from Annona cherimola. Phytochemistry 2001, 56, 753–757. [CrossRef][PubMed]

15. Pudjiantuti, P.; Mukhtar, M.R.; Hadi, A.H.A.; Saidi, N.; Morita, H.; Litaudon, M.; Awang, K. (6,7-dimethoxy-4-methylisoquinolinyl)-4′-methoxyphenyl)methanone, a New Benzylisoquinoline Alkaloid from Beilschmiedia brevipes. Molecules 2010, 15, 2339–2346. [CrossRef]

16. Katsui, N.; Sato, K.; Tobinaga, S.; Takeuchi, N. Alkaloids of lyciscion camtschatcense schott var. japonicum makino. Tetrahedron Lett. 1966, 7, 6257–6261. [CrossRef]

17. Rao, J.U.M.; Giri, G.S.; Hanumiaiah, T.; Rao, K.V.J. Sampangine, a New Alkaloid from Unonopsis lindmanii (Annonaceae). Phytochemistry 1979, 18, 800–806. [CrossRef]

18. Bowden, B.; Picker, K.; Ritchie, E.; Taylor, W. Constituents of Eupomatia species. IV. Structure and synthesis of eupalauridine (EL base-1) and some observations on the structures of eupolauramine (EL base-2) and hydroxyeupolauramine (EL base-3). Aust. J. Chem. 1975, 28, 2681–2701. [CrossRef]

19. Waterman, P.G.; Muhammad, I. Chemistry of the Annonaceae. Part 14. Sesquiterpenes and alkaloids from Cleistopholis patens. Phytochemistry 1985, 24, 523–527. [CrossRef][PubMed]

20. Mérienne, C.; Arango, G.J.; Cortes, D.; Cassels, B.K.; Cavé, A. Alkaloids of the Annonaceae. Part 80. Azafluorenones from Oxandra cf. major and biogenetic considerations. Phytochemistry 1987, 26, 2093–2098. [CrossRef]

21. El-Shanawany, M.A.; Slatkin, D.J.; Schiff, P.L.; El-Shabrawy, A. A new alkaloid from oxandra xylopioides diels. Bull. Pharm. Sci. Assiut 1985, 8, 127–143. [CrossRef]

22. Chakrabarty, M.; Patra, A. 6,7-Dimethoxyonychine and Other Alkaloids of Polyalthia longifolia. Indian J. Chem. Sect. B 1990, 21, 394–395. [CrossRef]

23. Chen, B.; Ye, Q.; Li, B.; Zhang, G. A new azafluorenone alkaloid from Miliusa balansae. Indian J. Heterocycl. Chem. 2002, 12, 81–82.

24. Yoshida, N.C.; De Siqueira, J.M.; Rodrigues, R.P.; Correia, R.P.; Garcez, W.S. An Azafluorenone Alkaloid and a Megastigmane Derivative and Antibacterial Activities of Alphonsea cylindrica Barks. Nat. Prod. Sci. 2017, 23, 151–156. [CrossRef]

25. Chaves, M.H.; Santos, L.D.A.; Lago, J.H.G.; Roque, N.F. Alkaloids from Porcelia macrocarpa. J. Nat. Prod. 2001, 64, 240–242. [CrossRef]

26. Talip, M.A.; Azziz, S.S.S.A.; Wong, C.F.; Awang, K.; Naz, H.; Bakri, Y.M.; Ahmad, M.S.; Litaudon, M. New Azafluorenone Derivative and Antibacterial Activities of Alphonsea cylindrica Barks. Nat. Prod. Sci. 2017, 23, 151–156. [CrossRef]

27. IUPAC. Compendium of Chemical Terminology, 2nd ed.; (The “Gold Book”); McNaught, A.D., Wilkinson, A., Eds.; Blackwell Scientific Publications: Oxford, UK, 1997; Online version (2019-) created by S.J. Chalk. Available online: https://goldbook.iupac.org/terms/view/R05360 (accessed on 27 October 2022).

28. NIST/EPA/NIH Mass Spectral Database (NIST17). Available online: https://chemdata.nist.gov/ (accessed on 27 October 2022).
29. Jourjine, I.A.P.; Bracher, F. Department of Pharmacy—Center for Drug Research, Ludwig Maximilians University Munich, Munich, Germany. 2023; manuscript in preparation.

30. Bracher, F. Polycyclic Aromatic Alkaloids. Part 2. Synthesis of Onychine and Eupolauridine. Arch. Pharm. 1989, 20, 293–294. [CrossRef]

31. Bracher, F. A regioselective Synthesis of Azafluorenone Alkaloids. Synlett 1991, 1991, 95–96. [CrossRef]

32. Bracher, F. Polycyclische aromatische Alkaloide, 10. Mitt.: Annonaceen-Alkaloide mit antymykotischer Aktivität. Arch. Pharm. 1994, 327, 371–375. [CrossRef] [PubMed]

33. Bracher, F. Polycyclische aromatische Alkaloide, I. Synthese von Cleistopholin und Sampangin. Liebigs Ann. Chem. 1989, 1989, 87–88. [CrossRef]

34. Plodek, A.; König, M.; Bracher, F. Synthesis of the Azaoxoaporphine Alkaloid Sampangine and Ascididemin-Type Pyridoacridines through TPMPMgCl·LiCl-Mediated Ring Closure. Eur. J. Org. Chem. 2015, 2015, 1302–1308. [CrossRef]

35. Bracher, F. Polycyclic aromatic alkaloids. Part 9: Total synthesis and anticandidal activity of diazafluoroanthene alkaloids. Pharmazie 1993, 48, 521–523. [PubMed]

36. Melzer, B.; Bracher, F. A divergent approach to benzylisoquinoline-type and oxoaporphine alkaloids via regioselective direct ring metalation of alkoxy isoquinolines. Org. Biomol. Chem. 2015, 13, 7664–7672. [CrossRef] [PubMed]

37. Puzik, A.; Bracher, F. A convenient approach to the canthin-4-one ring system: Total synthesis of the alkaloids tuboflavine and norisotuboflavine. J. Heterocycl. Chem. 2009, 46, 770–773. [CrossRef]

38. Bracher, F.; Hildebrand, D. β-Carbolin-Alkaloide, II Tributyl(1-ethoxyvinyl)stannan als C2-Baustein für die Synthese von β-Carbolin-Alkaloiden. Liebigs Ann. Chem. 1993, 1993, 837–839. [CrossRef]

39. Hetzler, B.E.; Trauner, D.; Lawrence, A.L. Natural product anticipation through synthesis. Nat. Rev. Chem. 2022, 6, 170–181. [CrossRef]