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Regiochemistry of Isoindolinone Formation in Stachybotrin-Type Alkaloids

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Abstract: Stachybotrin-type alkaloids are usually made rather than isolated. The standard route condenses a primary amine with the *ortho*-dialdehyde of stachybotrydial, closing the isoindolinone ring in one step. The substrate is unsymmetrical, though, and the ring can close through either aldehyde, so a single amine gives two lactams that differ only in whether C-8 or C-9 carries the carbonyl. For a long time this went unmentioned; products were reported without a regiochemical assignment. Work published in 2024 separated both isomers from tryptophan and tryptamine condensations and assayed them side by side, and the differences are not small. The C-8 isomers were the more cytotoxic against HepG2 and A549 cells, one of them from 2 micromolar upward, while the C-9 isomers were oxidised and glucuronidated faster in hepatic preparations. Within the tryptophan pair the C-9 isomer inhibited factor XIa by 66 per cent against 51 per cent for its partner, so the ranking of activity can invert with constitution alone. The isomer ratio responds to solvent, acid additive, drying agent and the rate at which the amine is delivered, which puts it within preparative reach. This paper reviews that evidence and sets out what a report of such a derivatization needs to contain.

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1. Introduction

Outside the laboratory *Stachybotrys* is known for trichothecenes and damp buildings. Its most abundant metabolites, however, are neither trichothecenes nor atranones but the phenylspirodrimanones, of which more than 120 have now been described and which are properly regarded as the dominant metabolite class of the genus [1]. Three structural groups are usually distinguished: tetracyclic compounds carrying alcohol or aldehyde functionality, with stachybotrydial as the parent; pentacyclic lactams and lactones such as stachybotrylactam; and the stachyflins, built on a *cis*-fused decalin [2]. The pharmacology is unusually broad for one structural class. Members of the family inhibit HIV-1 and influenza A at low micromolar concentrations [3], lower serum lipids [4], block osteoclast differentiation through the RANKL pathway [5], reverse ABCB1-mediated multidrug resistance and, in the case of stachybotrin C, protect neurons [6].

The lactams are both the interesting end of this family and the scarce one. Fermentation supplies the dialdehyde in workable quantity, whereas the individual lactams are minor constituents whose titres shift with the strain, the medium and the length of the cultivation [7]. Every group that has worked on the problem has answered it

in the same way: isolate the dialdehyde, add a primary amine, and assemble the lactam in the flask. One step, a substrate that is easy to obtain, and whatever side chain the amine happens to carry [8].

The awkward point is that the substrate is not symmetric. Its two aldehyde carbons sit in different surroundings, one next to the spiro quaternary centre and one next to the oxygenated aromatic position, and the five-membered ring can close through either. A single amine therefore gives two products, distinguished only by which carbon ends up as the carbonyl. For most of the history of this chemistry nobody said which one they had in hand. That changed in 2024, when both isomers of a tryptophan-derived and a tryptamine-derived lactam were separated, assigned and assayed together, and turned out to differ in cytotoxicity, in enzyme inhibition and in how quickly the liver disposes of them [9].

What follows starts from that result. The question here is not which substituent gives the best activity, which is a separate matter, but the ring closure itself: what decides the regiochemistry, how far it can be pushed, what an assignment actually rests on, and what a paper describing this reaction ought to report. Most of the evidence needed to answer this is already in the literature. It has simply not been collected in one place.

2. Materials and Methods

This is a survey of published work. No new experiments were carried out, and nothing is claimed here that the primary reports do not state.

Three bodies of literature were used. The first is the isolation record for the lactam-containing phenylspirodrimanes, from which the naturally occurring substitution patterns were taken: the stachybotrysins the chartarlactams the stachybotrin and stachyboicin congeners of the sponge-associated strain MUT 3308 stachybotrysin from a marine isolate and the deep-sea phenylspirodrimanes of Ma and co-workers. The second is the small semisynthetic literature in which an isoindolinone has been built deliberately from a phenylspirodrimane dialdehyde, namely the derivative library of Steinert and co-workers and the regioisomer study of Fischle and co-workers. The third supplies the controls: the total syntheses of stachybotrin C and of its enantiomer, which fix constitution and configuration without recourse to biosynthetic argument [10]; the crystallographic determination of absolute configuration through a heavy-atom derivative [11]; the LC-MS/MS survey of the metabolite class, which records how unstable the dialdehyde oxidation state is in practice and the general study of *N*-capping of primary amines by 2-acylbenzaldehydes, which is the mechanistic template for the case at hand [12].

Each paper was read with the same questions in mind. In what protection state was the substrate used? What class of amine? What solvent, additive and mode of addition? One product or a mixture? On what evidence was the regiochemistry assigned? And what difference, if any, was reported between the isomers? Where a report says nothing about regiochemistry, that silence is recorded as such and not filled in by inference.

3. Results and Discussion

The mechanism locates the selectivity problem in one particular place. A primary amine adds to one of the two aldehyde carbons to give a hemiaminal, which loses water to an iminium ion; the remaining aldehyde is then attacked from within the molecule, and an oxidation-state adjustment delivers the lactam. The carbon attacked first becomes the methylene; the carbon attacked second becomes the carbonyl. Everything is therefore decided at the first addition, and that addition is reversible. Augner and co-workers characterised the general reaction, condensing primary amines with 2-acylbenzaldehydes to give isoindolinones cleanly. There, though, the two carbonyls are an aldehyde and a ketone, and the gap in reactivity between them is wide enough to force one outcome. In stachybotrydial and its acetates both are aldehydes, separated only by their surroundings. Nothing in the substrate enforces a choice, which is exactly why two products should be

the default expectation, and also why the outcome is open to manipulation. Jagels and co-workers add a further complication: they observed spontaneous dialdehyde-lactone isomerisation among these metabolites during analytical work so the substrate is already moving between electrophilic forms before any amine reaches it.

Both isomers have now been isolated. Fischle and co-workers condensed acetoxystachybotrydial acetate with L-tryptophan in acetonitrile at room temperature for about four hours in the dark, and with tryptamine under a different protocol, and in each case recovered two lactams rather than one: an A-type bearing the carbonyl at C-8 and a B-type bearing it at C-9 [13]. The pair could be told apart by retention behaviour, by characteristic differences in the ultraviolet spectra, by NMR and by mass-spectrometric fragmentation, and the assignment rests on that combination rather than on any one method. This has a bearing on what came before. The derivative library of Steinert and co-workers was built on the same platform and supplied the agmatine-derived lactam that the later study used as a reference compound but it predates the recognition of the pair, as does the whole isolation literature in which a natural lactam is described without reference to the alternative constitution. None of that work becomes wrong. It does mean that a single reported product in this family cannot be assumed to be the only one formed, or even the major one.

Biologically the two are not interchangeable, and this is what makes the question worth raising. In the resazurin assay against HepG2 and A549 cells the A-type isomer was the more toxic member of each pair, the tryptamine-derived A-type compound showing pronounced cytotoxicity in HepG2 cells from 2 micromolar upward, while both B-type isomers were appreciably weaker; neither type induced micronuclei anywhere in the 0.5 to 25 micromolar range tested. Against a panel of serine proteases the same variable reorders the compounds instead of merely scaling them. The tryptophan-derived pair was far more active than the tryptamine-derived pair, which was essentially inactive, and within that pair the B-type isomer was the better inhibitor of factor XIa, at 66 per cent against 51 per cent, and inhibited factor XIIa by 63 per cent [14]. Metabolism points the same way: B-type compounds underwent faster phase I oxidation and were more readily glucuronidated, so within these series the less cytotoxic constitution is also the more rapidly cleared one. Two compounds of identical molecular formula, from the same amine and the same substrate, thus differ in potency, in which enzyme they prefer, and in how long they survive hepatic metabolism.

The ratio between them can be shifted, which is the practical part of the story. The two condensations reported by Fischle and co-workers were not run alike. Tryptophan reacted in acetonitrile at room temperature; tryptamine needed a deliberately engineered protocol, in dimethyl sulfoxide containing glacial acetic acid at 100 micromolar, over 3 angstrom molecular sieve, with the amine delivered by syringe pump at 10 microlitres per minute for two hours and a further hour of stirring, all in the dark. Every element of that arrangement acts on the first addition rather than on the ring closure. The acid catalyses iminium formation, the sieve removes the water that would reverse it, and slow delivery keeps the amine concentration low, so that the small intrinsic difference between the two aldehydes is not swamped. That such a protocol was needed at all is the clearest indication available that the outcome is kinetic and therefore tunable.

Protection belongs to the same discussion. Both semisynthetic studies used the doubly acetylated dialdehyde [which masks the phenolic and alcoholic oxygens that would otherwise compete as nucleophiles and, in doing so, alters the electronic environment of the neighbouring aldehyde. The fungus uses the same devices: stachybotrylactam acetate and 2- α -acetoxystachybotrylactam acetate occur as natural congeners beside the free lactam and stachybotrysin D is a naturally occurring alcoholic O-sulfate. O-functionalisation is thus a real axis of variation in this family and not a synthetic artefact.

Finally, the analytical burden should be stated plainly, since an assignment of this kind comes down to locating a carbonyl. Synthetic material now exists to check against. Jacolot and co-workers prepared stachybotrin C together with all four stereoisomers and revised the published structure along the way and Xie and co-workers reached *ent*-stachybotrin C and a congener in a twelve-step longest linear sequence whose key operation is a Knoevenagel condensation, Hantzsch ester reduction, epoxide opening and transesterification cascade. Where a crystal can be grown, heavy-atom derivatization is still decisive: Kuroda and co-workers settled the (8*S*,9*R*) configuration of stachybotrin C through its di(4-bromobenzyl) ether after the corresponding dimethyl ether proved inadequate [15].

4. Conclusion

The amine-mediated closure of an isoindolinone ring onto a phenylspirodrimane dialdehyde is the most productive reaction in stachybotrin chemistry, and it has an unresolved degree of freedom at its centre. Because the two aldehyde carbons are inequivalent but not strongly differentiated, and because selectivity is settled at the first reversible addition, the reaction yields two constitutional isomers whose ratio depends on solvent, water activity, acid additive and how quickly the amine is supplied. The isomers are not interchangeable. The carbonyl-at-C-8 constitution is the more cytotoxic; the carbonyl-at-C-9 constitution is oxidised and conjugated faster; and which of the two inhibits a given protease better depends on the constitution and not on the amine alone.

For preparative work this means that the conditions governing the first addition are the levers worth optimising, and that they should not simply be inherited from whatever substrate was used last. For characterisation it means locating the carbonyl explicitly, through correlation spectroscopy supported by fragmentation behaviour and, where a crystal can be had, by crystallography on a heavy-atom derivative or by comparison with synthetic material. And for reporting it means that the isomer ratio is part of the result: a yield quoted for an unspecified lactam is not reproducible information in this family.

Two questions follow directly. Can catalysis, whether by small organic catalysts or by enzymes, enforce selectivity for one aldehyde over the other and turn a tunable ratio into a single clean product? And do the acetylation and sulfation patterns that occur naturally in this family bias the ring closure? An affirmative answer to the second would suggest that the fungus already exercises the control the chemist is still approximating.

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