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TETRAHEDRON

Synthesis of 6-Azido-1-oxo-indan-4-oyl Isoleucine; a Photoaffinity Approach to Plant Signaling[1]

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[1]Dedicated to Prof. Dr. Fritz Vögtle on the occasion of his 60th birthday

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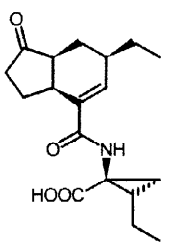
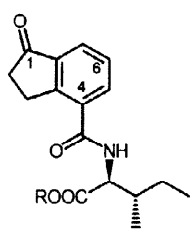
Abstract: The synthesis of the photoreactive 6-azido-1-oxo-indanoyl isoleucine (**3**), as a molecular probe for the identification of putative receptors and binding proteins involved in stress signaling of plants, is described. The biological activity of the photolabile azide **3** comes close to that of the bacterial phytotoxin coronatine (**1**). Photodecomposition of **3** in the presence of myoglobin is assumed to proceed via an didehydroazepine intermediate and results in significant binding of the probe to the model protein. © 1999 Elsevier Science Ltd. All rights reserved.

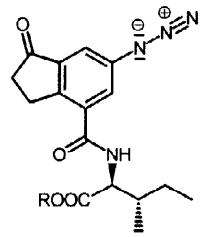
In recent years the phytotoxin coronatine (**1**) has attracted considerable interest, since **1** mimics many of the biological activities generally associated with jasmonic acid (**4**), one of the most powerful low molecular signaling compounds involved in plant stress responses.[1,2]



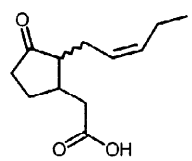
coronatine **(1)**



1-oxo-indanoyl isoleucine **(2)**
R = H, CH_3



6-azido-1-oxo-indanoyl isoleucine **(3)**
R = H, CH_3



jasmonic acid **(4)**

Coronatine (**1**), is a conjugate of coronafacic acid with the rare cyclopropyl amino acid coronamic acid. The phytotoxin is produced by several pathovars of *Pseudomonas syringae* (e.g. *tomato*, *glycinea*, *atropurpurea*)[3] and was first isolated by Ichihara et al. in 1978 from a fermentation broth of *P. syringae* var. *atropurpurea*.[4] Application of **1** to higher plants elicits, among many other responses,[1] tendril coiling in *Bryonia diocia* (mechanoreception)[2] and volatile biosynthesis,[5] which represents a typical stress response after herbivore feeding.[6] The structurally much simpler, and accordingly designed, synthetic analogue 1-oxo-indanoyl-isoleucine (**2**) is also a powerful elicitor of volatile biosynthesis and can be used almost without exception instead of the difficult to access coronatine (**1**).[7] Systematic studies with different amino acid conjugates of **2** recently showed that different receptors or other elements must exist in the signaling chain, since different volatile patterns could be provoked in leaves of the Lima bean (*Phaseolus lunatus*) by simply changing the amino acid moiety. A similar diversity has been observed in the gene expression of tomato leaves when treated with various octadecanoids and jasmonates.[8] Owing to this finding, synthesis of the photolabile 6-azido derivative of **3** appeared to be a promising strategy to address the identification of unknown biological targets by photoaffinity

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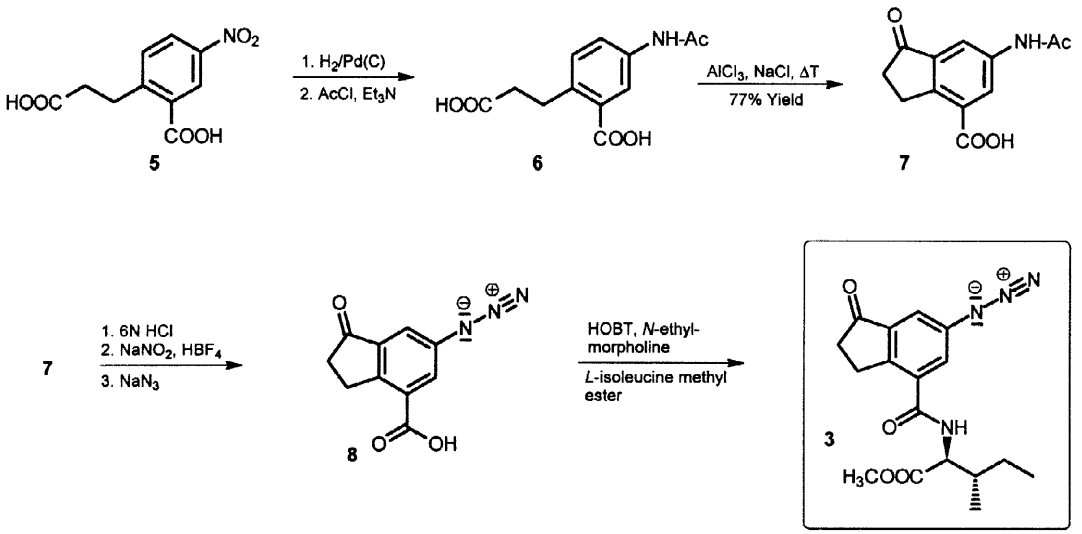
labelling.[9] By analogy with highly active coronatine the photoreactive group should reside on C(6), since previous structure-activity studies revealed significant restrictions concerning substitution pattern and ring size.[10] Since their introduction by Knowles[11] in 1969 arylazides have been used with great success to selectively tag the active sites of proteins with radioactive or fluorescent affinity labels.[12] Placing a photolabile azide at C(6) would *i*) preserve the non-polar character of the ethyl substituent of **1** and *ii*) not noticeably increase the size of the ligand, thus guaranteeing maximum similarity of the steric and electronic properties of coronatine (**1**) and the photolabile affinity probe **3**. Moreover, the 6-azido-1-oxo-indan-4-carboxylic acid could serve as a central building block which could be combined with a great variety of amino acids to create a library of photolabile probes, probably endowed with different affinities to allow the tagging of different receptors and different binding proteins. Here we report the synthesis of 1-oxo-indanoyl-isoleucine (6-azido-IN-Ile) and provide first data on the biological activity and on the photodecomposition products of the novel affinity probe.

Synthesis of 6-Azido-1-oxo-indanoyl Isoleucin (3).- The synthesis of the photolabile isoleucine conjugate **3** was accomplished according to the protocol outlined in Scheme 1. Since the direct functionalization, e.g. nitration or bromination, of the readily available 1-oxo-indan-4-carboxylic acid[13] failed due to the strong deactivation of the aromatic nucleus by the two adjacent carbonyl functions, the 5-nitrodicarboxylic acid **5**, was chosen to build up the indanone skeleton by intramolecular Friedel-Crafts acylation.[14] **5** possesses the correct substitution pattern and is available as a single isomer by nitration of 2-(2-carboxy-ethyl)-benzoic acid.



First attempts to carry out the intramolecular Friedel-Crafts acylation with **5** failed owing to the strong deactivating effect of the nitro group. However, after reduction and acylation, the resulting amide **6** allowed a smooth cyclization upon heating with $AlCl_3$/NaCl and furnished the acid **7** in high overall yield from **5**. The amide was cleaved by acid hydrolysis (6N HCl), and the resulting ammonium salt was directly diazotated and decomposed in the presence of sodium azide. Subsequent conjugation of the 6-azido acid **8** with L-isoleucine proceeded without difficulty following our previously established route.[7,14] The affinity probe **3** was obtained in good yield and proved to be stable in the absence of direct light.

Photolysis of the 6-Azido Conjugate.- The UV spectrum of **3** showed two absorption maxima at 273 nm and 322 nm, respectively (cf. Figure 1, insert). Photochemical experiments were carried out with solutions of **3** in THF, in the presence of diisopropylamine, in a standard UV cuvette (d = 1 cm) using a low power detection

lamp at 254 nm (6 W) for irradiation. Diisopropylamine served as a scavenger to trap the very short-lived, reactive didehydroazepine intermediates **10a/b**.[15]

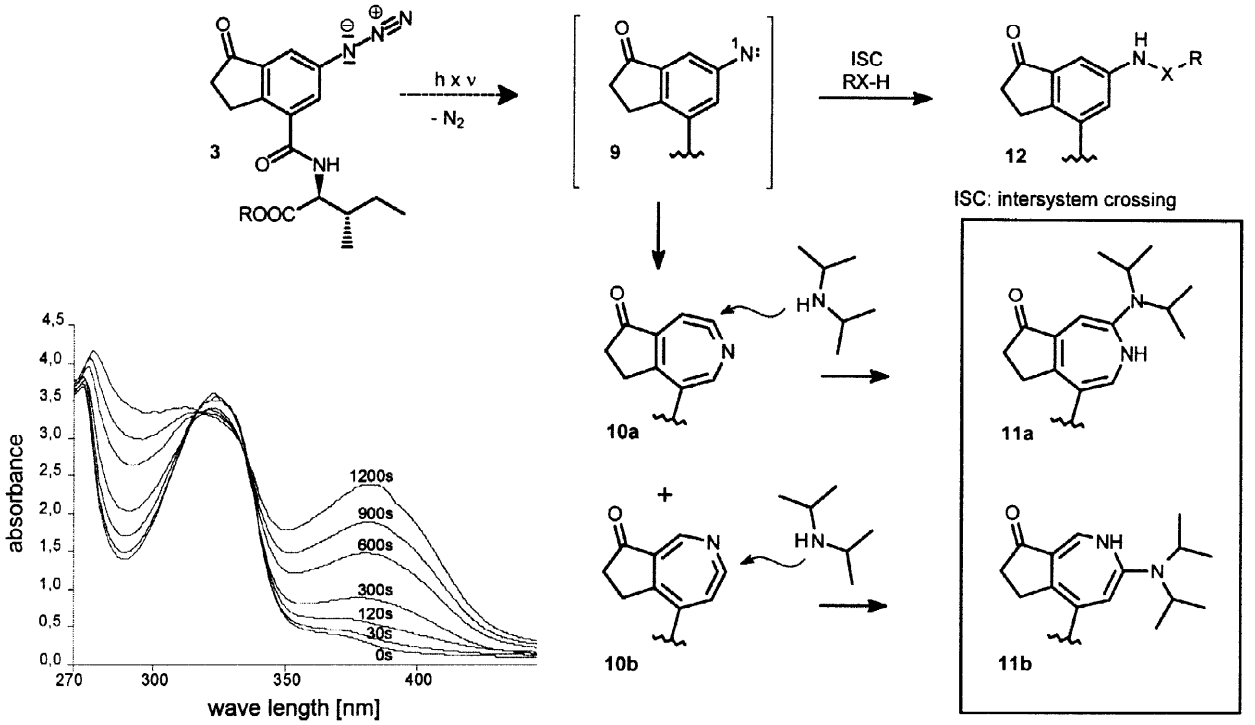


Figure 1. Photodecomposition and reaction products of **3** obtained in the presence of diisopropylamine as a nucleophile to trap the unstable didehydroazepine intermediate. Inset: Time course of the photolysis of **3** monitored by UV.

In accord with previous results,[9,15,16] irradiation of the aryl azide **3** was expected to generate its singlet excited state which may loose nitrogen to form the singlet nitrene **9**. Among other reactions, the latter may suffer rapid ring expansion to the isomeric didehydroazepines **10a/b**. In the presence of a nucleophile like, for example, diisopropylamine the reactive intermediate **10a/b** should be trapped yielding the isomeric 3*H*-azepines **11a/b**. Intersystem crossing of the exited singlet azide will lead to a triplet azide which, after the loss of nitrogen, will furnish a triplet nitrene able to insert into appropriate RX-H bonds (X=O,N) via radical intermediates (cf. **12**). Preparative scale photolytic experiments confirmed the general pathway outlined in Figure 1 and afforded the expected 3*H*-azepines **11a/b** in moderate yield (ca. 42%) after chromatography. As the isomers could be not separated or distinguished by spectroscopy, the ratio of **10a/b** is not known. The course of the reaction was monitored by the appearance of a new absorption at 400 nm and showed a half life time for **3** of 790 sec at rt. ($k = 0.877\ 10^{-3}\ sec^{-1}$). Attempts to characterize products of a triplet nitrene intermediate as their corresponding trifluoroacetamides[15] remain unsuccessful.

To demonstrate the ability and the ease of covalent labeling of macromolecular targets with reactive intermediates from photodecomposition of **3**, an aqueous solution of myoglobin was irradiated in the presence of a large excess of the azide (see Experimental). MALDI-TOF analysis of the crude reaction products revealed the presence of significant amounts of mono-, bis- and tris-adducts of **10a/b** and myoglobin (summed to 100%): myoglobin $[M+H]^+$ 16961 (45%), myoglobin-monoadduct $[M+H]^+$ 17294 (34%); myoglobin-bisadduct $[M+H]^+$ 117617 (15%); myoglobin-trisadduct $[M+H]^+$ 117948 (6%) .

Biological Activity of the 6-Azido Conjugate (3).- When freshly cut plantlets of fourteen-day-old Lima beans were placed into an aqueous solution containing the azido conjugate **3** (100.0 μM), a significant *de novo* biosynthesis of predominantly terpenoid volatiles was induced. Compared to the unsubstituted indanone conjugate **2** the azido derivative was about twenty fold more active coming close to the threshold concentration reported for coronatine-mediated volatile induction (ca. 5-10 μM).[5] Moreover, the pattern of induced volatiles showed, besides the typical jasmonate-responsive compounds (Figure 1), the presence of the C_{16} homoterpene, 4,8,12-trimethyltrideca-1,3,7,11-tetraene (TMTT) which, in the Lima bean, was not induced by jasmonic acid treatment.[5] According to recent findings the biosynthesis of the degraded diterpene TMTT is triggered in the Lima bean only by early intermediates of the octadecanoid-signaling pathway like, for example, phytodienoic acid or its structural analogue coronatine (**1**).[8] A conjugate of 1-oxo-indan-4-carboxylic acid with the cyclopropyl amino acid of coronatine (coronamic acid) had the same effect,[10] suggesting, that the selectivity of the photoaffinity approach can be individually tailored to address different macromolecular targets. Preliminary experiments with **3** and tendrils of *Bryonia dioica* showed that the azide also triggers mechanoreceptors[2] inducing tendril coiling.

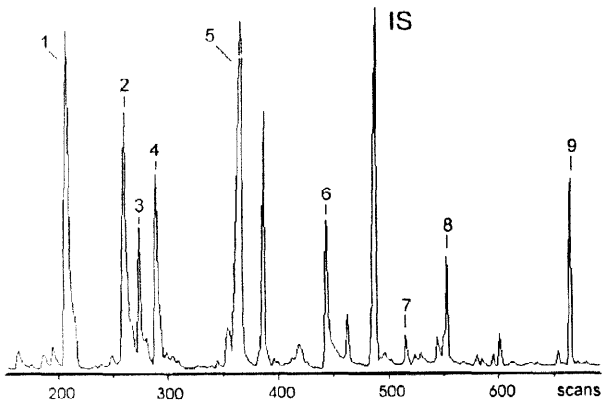


Figure 1. Pattern of the induced volatiles after elicitation of Lima bean leaves with the 6-azido conjugate **3**. Freshly cut plantlets from fourteen-day-old Lima beans with two fully developed leaves were placed into an aqueous solution containing **3** at 100.0 μM. Volatiles were collected during 48 h by CLSA[17] or SPME.[18] Identification of compounds: (1) *cis*-ocimene, (2) linalool, (3) 4,8-dimethylnona-1,3,7-triene (DMNT), (4) $C_{10}H_{14}$, (5) $C_{10}H_{14}O$, (6) indole, IS = internal standard, (7) α-copaene, (8) β-caryophyllene, (9) 4,8,12-trimethyltrideca-1,3,7,11-tetraene (TMTT).

Although the unsubstituted indanoyl conjugate **2** failed to induce jasmonate responsive gene expression when applied to barley leaves,[7,19] the azide **3** clearly triggered the expression of the gene coding for the jasmonate-inducible-protein of 23 kDa (JIP23) when applied at higher concentrations (100μM, lane 4, Figure 2). However, unlike the induction of volatile biosynthesis in the Lima bean (Figure 1), in the barley system methyl jasmonate was significantly more active than azide **3**.

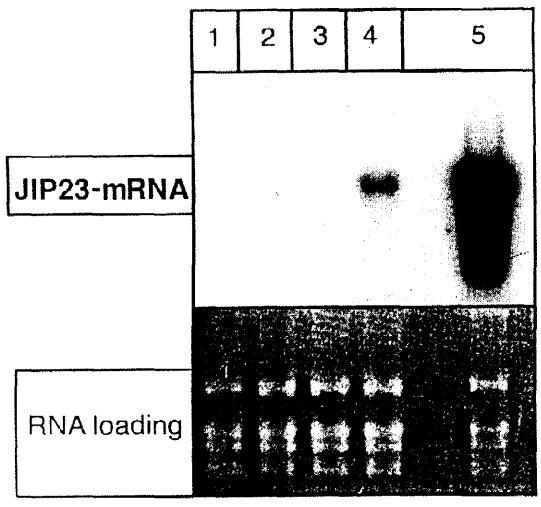


Figure 2. Nothern blot analysis of accumulation of mRNA coding for JIP23 in barley.[20] Segments of primary leaves of light-grown seedlings were floated on water (lane 1), azide 3 at 10 μM (lane 2), at 45 μM (lane 3), at 100 μM (lane 4) and (±)-jasmonic acid methyl ester at 45 μM (lane 5) in darkness for 24 h. 10 μg total RNA of leaf segments were loaded per lane. RNA loading is given below.

Experimental

General: Reactions were performed under Ar; solvents were dried according to standard methods. UV: Perkin-Elmer Lambda 16. IR: Bruker Equinox 55 FTIR Spectrophotometer. ^{1}H- and ^{13}C NMR: Avance DRX 500 spectrometer; $CDCl_3$ as solvent. Chemical shifts of ^{1}H and ^{13}C NMR are given in ppm (δ) downfield relative to TMS as internal standard. GC-MS (70eV): Finnigan GCQ, equipped with a fused silica, coated with DB1 or SE 30 (15m x 0.31mm); helium served as carrier gas. MALDI-TOF: Micromass TOF-Spec 2E with N_2-laser at 337 nm. HR-MS: Kratos MS 50. Silica gel, Si 60 (0.200-0.063 mm, E. Merck, Darmstadt, Germany) was used for chromatography. Thin layer chromatography was performed with silica gel plates from Kodak, Rochester, N.Y.. UV-lamp for Photolysis: Konrad Benda (Wiesloch, Germany) NU-6 KL, 2x6 Watt, 254 and 366 nm.

5-Acetylamino-2-(2-carboxy-ethyl)-benzoic Acid (6)

5-Nitro-2-(2-carboxy-ethyl)-benzoic acid **5** (2.0 g, 8.6 mmol)[14] was hydrogenated in aqueous NaOH (0.6 M, 75 ml) under atmospheric pressure using Pd/C (10%, 0.10 g) as the catalyst. After stirring for 18h the catalyst was removed by filtration and the solution neutralised with HCl. The resulting precipitate was filtered off and dried. Then, the crude product was suspended in ether (20 ml) and triethylamine (2.0 ml) and stirred at 0 °C while acetyl chloride (0.60 ml, 8.6 mmol) was added slowly. Stirring was continued for 1h at 0 °C and the suspension was allowed to come to r.t. and stirred for another 30 min. The colourless solid was collected by filtration, washed with water (3 x 5.0 ml) and dried. Yield: 1.50 g (85%). M.p.: 119-122 °C. ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 2.09 (s, 3H-CH_3); 2.53 (t, *J*=7.6, 2H-C(2')); 3.11 (t, *J*= 7.6, 2H-C(1')); 7.30 (d, 1H-C(3)); 7.71 (d, 1H-C(4)); 8.09 (s, 1H-C(6)); 10.06 (s, NH). ^{13}C NMR (DMSO-d_6, 125 MHz) δ: 23.9 (Me), 28.6 (C-1'), 35.4 (C-2'), 120.8 (C-6), 122.2 (C-4), 130.4 (C-3), 131.1 (C-1), 136.3 (C-2), 137.5 (C-5), 168.4 (CONH), 170.6 (Ar-COOH), 173.8 (COOH). IR (KBr): 3301, 2976, 2677, 2491, 1731, 1704, 1630, 1606, 1552, 1504, 1414, 1394, 1291, 1260, 899, 827 cm^{-1}. MS (70 eV): 251($M^{+\bullet}$, 14), 233(7), 205(45), 163(65), 150(100), 146(11), 118(7), 94(12), 77(10). HR-MS: *m/z* calcd. for $C_{12}H_{13}NO_5$: 251.0794, found: 251.0787.

6-Acetylamino-1-oxo-indan-4-carboxylic Acid (7)

5-Acetylamino-2-(2-carboxy-ethyl)-benzoic acid (**6**) (1.40 g, 4.5 mmol) was thoroughly mixed with anhydrous aluminium chloride (20.0 g) and sodium chloride (3.0 g). The mixture was heated for 2h to ≈140 °C and the resulting dark, viscous liquid was stirred occasionally. After cooling the complex was hydrolysed with ice water (10 ml) and 6N hydrochloride acid (30 ml) and stirred for several hours at rt. The solids were collected by filtration, washed thoroughly with water and dried. Yield: 1.0 g (77 %). ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 2.06 (s, 3H-CH_3); 2.63 (m, 2H-C(3)); 3.29 (m, 2H-C(2)); 8.17 (s, 1H-C(5)); 8.34 (s, 1H-C(7)) and 10.4 (s, 1H-NH), 13.2 (s br, 1H-COOH). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 23.9 (CH_3), 26.4 (C-3), 36.0 (C-2), 116.1 (C-6), 126.8 (C-4), 129.0 (C-7a), 138.4 (C-5), 138,9 (C-7), 150.5 (C-3a), 166.6 (COOH), 168.7 (CONH) and 205.8 (C-1). IR (KBr): 3295, 2920, 2595, 1718, 1690, 1653, 1609, 1545, 1479, 1430, 1373, 1305, 1231, 1206, 1141, 897, 714 cm^{-1}. MS (70 eV): 233($M^{+\bullet}$, 50), 191(100), 163(11), 146(45), 135(10), 106(9), 89(8). HR-MS: *m/z* calcd. for $C_{12}H_{11}NO_4$: 233.0688, found: 233.0686.

6-Azido-1-oxo-indan-4-carboxylic Acid (8)

A suspension of 6-acetylamino-1-oxo-indan-4-carboxylic acid (**7**) (0.90 g, 3.9 mmol) in 6N hydrochloric acid (5.0 ml) was refluxed with stirring for 2h. After evaporation to dryness *in vacuo*, the residue was suspended in

fluoroboric acid (3.0 ml, 50%-soln.). The chilled and well stirred solution was gradually treated with a solution (0.60 ml) of sodium nitrite in water (0.50 g / ml). The resulting solid was isolated by filtration and resuspended in water (2.0 ml). The suspension was vigorously stirred and a solution of sodium azide (0.30 g) in water (0.60 ml) was added slowly. The yellow precipitate was isolated by filtration and washed with water. Yield: 0.66 g (78%). M.p.: 175-177 °C (decomp.). ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 2.47 (t, 2H-C(3)), 3.07 (t, 2H-C(2)), 7.43 (s, 1H-C(5)), 7.73 (s; 1H-C(7)). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 27.4 (C-3), 37.0 (C-2), 117.4 (C-6), 127.3 (C-4), 131.2 (C-8), 140.3 (C-5), 140.4 (C-7), 153.4 (C-9), 166.9 (COOH) and 206.0 (C-1). IR (KBr) ν_{max}: 3066, 2979, 2935, 2127 (N_3), 1725, 1696, 1609, 1482, 1420, 1311, 1238, 1169 cm^{-1}. MS (70 eV): 217($M^{+\bullet}$, 20), 189 (100), 161(22), 133(22), 116(11), 105 (15), 89(28), 63(23). HR-MS: *m/z* calcd. for $C_{12}H_{11}NO_4$: 217.0487, found: 217.0488.

2-[(6-Azido-1-oxo-indan-4-carbonyl)-amino]-3-methyl-pentanoic Acid Methyl Ester (3)

A chilled and well stirred solution of 6-azido-1-oxo-indan-4-carboxylic acid (**8**) (0.10 g, 0.4 mmol), methyl ester of L-isoleucine hydrochloride (0.083 g), 4-ethylmorpholine (0.053 g) and 1-hydroxybenzotriazole (HOBT) (0.115 g) in THF (5.0 ml) was gradually treated with *N,N'*-dicyclohexylcarbodiimide (0.10 g). Stirring was continued for 20 h at 0°. The precipitated urea was removed by filtration and the solvent was evaporated *in vacuo*. The resulting solid was redissolved in ethyl acetate (5.0 ml) and the organic layer was washed three times with 2N HCl (5.0 ml) and sat. aq. $NaHCO_3$ (5.0 ml) and dried with $MgSO_4$. After removal of solvent *in vacuo,* the isoleucine conjugate was purified by chromatography on silica gel (ethyl acetate:hexane, 1:2, v/v). Yield: 0.110 g (82%). M.p.: 112-114 °C. ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 0.94 (t, *J*=7.4, 3H-C(5)), 0.98 (d, *J*=6.8, Me-C(3)), 1.34 (dq, *J*=7.4 and 22.0, H-C(4)), 1.50-1.60 (m, H-C(4)), 1.96-2.03 (m, 1H-C(3)), 2.74 (t, *J*=5.7, 2H-C(2')), 3.21-3.33 (m, 2H-C(3')), 3.74 (s, MeO-C(1)), 4.47 (t, 1H-C(2)), 7.47 (d, $J_{1,3}$=2.1, 1H-C(5')), 7.65 (d, $J_{1,3}$=2.1, 1H-C(7)), 8.86 (d, NH). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 11.0 (C-1), 15.5 (Me-3), 25.1 (C-4), 25.2 (C-3'), 35.9 (C-3), 36.2 (C-2'), 51.7 (MeO-1), 57.1 (C-1), 114.4 (C-6'), 124.4 (C-4'), 134.6 (C-7a'), 138.9 (C-5'), 139.2 (C-7'), 150.5 (C-3a'), 166.1 (CO-4'), 171.9 (C-1) 205.2 (C-1'). IR (KBr): 2964, 2932, 2858, 2116 (N_3), 1746, 1728, 1707, 1636, 1523, 1465, 1340, 1311, 1235, 1197 cm^{-1}. MS (70 eV): 344($M^{+\bullet}$, 5), 316(35), 285(10), 256(100), 227(10), 200(25), 188(10), 171(30), 143(33), 116(12), 99(11), 89(9), 69(9). HR-MS: *m/z* calcd. for $C_{17}H_{20}N_4O_4$: 344.1485, found: 344.1489.

Photolysis of the Azide (3) and Identification of Reaction Products

A solution of the azide **3** (0.49 mg, 1.42 μmol) in THF (1.4 ml) was treated with diisopropylamine (19.0 μl, 136 μmol) and irradiated at rt. in quartz cell (d = 1 cm) with UV light (6 W DC-Detection lamp) at 254 nm. The progress of the reaction was monitored by the increase of UV absorption of the photoproducts at 400 nm. Under the above conditions the azide **3** had a half life time of 790 sec (k = 0877 x 10^{-3} sec^{-1}). Preparative scale photolytic experiments were carried out with larger amounts of **3** (5.88 mg, 17.04 μmol) and diisopropylamine (228 μl, 1.63 μmol) in THF (20 ml) under otherwise identical conditions for several hours of irradiation (254 nm) until complete decomposition of the azide was indicated by the UV spectrum (cf. Figure 1). TLC (ethyl acetate) indicated the presence of three products. After removal of solvents the main product (R_f = 0.43) was obtained by chromatography on silica gel (ethyl acetate for elution) as a brown solid. Yield: ca. 3 mg

(42%). ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 0.95(m, H-(CH_3)), 0.98 (t, 3H-C(5)), 1.26 (m, H-CH_3), 1.23 and 1,51 (m, H-C(4)), 1.97 (m, H-C(3)), 2.70 (t, H-C(7')), 3.27 (t, H-C(8')), 3.77 (s, H-CH_3O), 4.13 (dq, H-CH), 4.33 (m, H-NH), 4.71 (m, H-C(2)), 6.23 (d, H-NH), 7.11 (d, *J*=2.08, H-C(5')), 7.28 (d, *J*=2.08, H-C(2')). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 11.6 (CH_3-3), 15.6 (C-5), 25.3 (C-4), 25.5 (C-8'), 36.3 (C-7'), 38.2 (C-3), 52.1 (CH_3O-1), 57.1 (C-2), 106.3 (C-4'), 110.7 (C-5'), 120.8 (C-2'), 143.2 (C-5a'), 150.4 (C-8a'), 164.7 (CO-1'), 168.6 (C-1'), 172.7 (C-1), 206.0 (C-6'). IR (KBr): 2964, 2917, 2848, 1741, 1676, 1636, 1558, 1487, 1371, 1250, 1205, 1140, 1038 cm^{-1}. MS: 417($M^{+\bullet}$, 45), 374(100), 314(5), 273(15), 229(83), 189(13), 162(22), 134(11), 100(5). HR-MS: *m/z* calcd. for $C_{23}H_{35}N_3O_4$: 417.2628, found: 417.2622.

Photodecomposition of 3 in the Presence of Myoglobin

A solution of **3** (4.0 mg, 11.6 μmol) in water (10 ml) was mixed with 50 μl of myoglobin (ca. 24 nmol) in water (0.4 mg in 0.1 ml) and irradiated with UV light (254 nm, 6W) for 20 min. The solvent was removed *in vacuo* and the resulting solid was analyzed by MALDI-TOF. Bovine trypsinogen and cytochrome C (equine) were added as internal calibration standards. Sinapinic acid served as the matrix. MS (MALDI-TOF): (relevant peaks summed to 100%): myoglobin $[M+H]^+$ 16961 (45%), myoglobin-monoadduct $[M+H]^+$ 17294 (34%); myoglobin-bisadduct $[M+H]^+$ 117617 (15%); myoglobin-trisadduct $[M+H]^+$ 117948 (6%). Analysis of a control experiment, performed with myoglobin under identical conditions but without addition of the azide **3** revealed only $[M+H]^+$ for myoglobin and minor matrix-protein adduct ions.

Induction of Volatile Biosynthesis by the Azide (3)

Owing to the limited solubility of the photoprobe, a stock solution of the azide (0.34 mg) in DMSO (15 μl) was dissolved in water (10 ml) with rapid stirring until a clear solution resulted. A freshly detached plant with two fully developed leaves of a fourteen-day old Lima bean (*Phaseolus lunatus*) was placed into the solution (2.0 ml in small screw capped vial). The vial was then enclosed in a small desiccator (250 ml), and after 24 h the volatiles were collected on charcoal (1.5 mg, CLSA Filter, Le Ruisseau de Montbrun, F-09350 Daumazan sur Arize, France) as described.[17] Following desorbtion of the carbon traps (2 x 20 μl CH_2Cl_2 and addition of bromodecane as internal standard) the volatiles were analysed by GLC-MS. Alternatively, an SPME-fiber (Supelco Inc.),[18] coated with 100 μm polydimethylsiloxane was introduced into the desiccator through a tightly fitting whole in a teflon stopper, and the fibre was exposed to the atmosphere for ca. 15 min. The fiber was inserted into the injection port of the gas chromatograph and, after evaporation of the absorbed volatiles, the compounds were separated under temperature programmed conditions and identified by their mass spectra. Finnigan GCQ 800 mass spectrometer equipped with a fused silica, coated with DB 5 (15 m x 0.32 mm). Carrier gas: He. Temperature program: 50°C for 1 min, then at 10 °C min^{-1} to 200°C, followed by 35 °C min^{-1} to 280°C. Transfer line: 260°. Ion source: 220°. For identification of compounds see Figure 1.

RNA Extraction and Nothern Blot Analysis of Azide-induced Barley Leaves

Primary leaves of 7-day-old seedlings of barley (*Hordeum vulgare* cv. Salome) were used to detect the most prominent gene expression in response to jasmonates, the synthesis of the jasmonate-inducible-protein of 23 kDa (JIP 23). Growth of seedlings, RNA extraction and Northern blot analysis were performed as described.[20] Due to the fact that the azide **3** is degraded by light, all treatments by floating the leaf segments on the respective compounds were performed in the darkness.

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