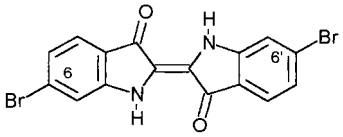
Akashins A, B, and C: Novel Chlorinated Indigoglycosides from *Streptomyces* sp. GW 48/1497**

Rajendra P. Maskey, Iris Grün-Wollny, Herbert H. Fiebig und Hartmut Laatsch*

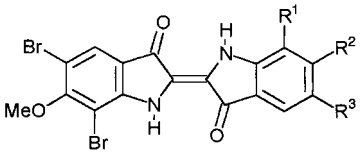
Dedicated to Professor Wolfgang Lüttke

Indigo and its dibromo derivative, purple **1a** are amongst the most important dyes. They were already used in a ancient civilizations[1] and indigo is still an industrially important dye. Numerous derivatives have been synthesized, not only for dyeing textiles, but also for important theoretical investigations of the chromophoric properties of indigo. The pigment



1a

1b: 5,5'-Cl instead of 6,6'-Br



2a: R^1, R^3 = H, R^2 = Br

2b: R^1 , R^3 = Br, R^2 = OMe

can be obtained from various higher plants such as *Baphicacanthus cusia* (Acanthaceae), *Calanthe veratrifolia* (Orchidaceae), *Isatis tinctoria* (Brassicaceae), and *Polygonum tinctorium* (Polygonaceae), by a process that involves its formation from precursors such as indican and isatan. Indigo has also been isolated from fungi (*Schizophyllum commune*,[2] *Agaricus campester*[3]) and has even been detected in the urine, blood plasma, and haemofiltrate of patients[4] suffering from metabolic disorders (Blue-Diaper syndrome). Whereas numerous derivatives of naturally occurring chromophores are also found in nature, only three derivatives of the blue indigo isomer (indigotin) occur: in addition to the well-known Tyrian purple (6,6′-dibromindigo, **1a**)[5] from the purple snail, only derivatives **2a** and **2b** from the marine invertebrate *Ptychodera flava laysanica* are known.[6]

We have now isolated derivatives of 5,5′-dichloroindigo (**1b**) from the terrestrial *Streptomyces* sp. GW 48/1497. The chromophore **1b** was previously unknown as a natural product. The derivatives are unsymmetrically monoglycosylated at the N1 atom of **1b**. Even synthetic N-glycosides of indigo have not been described previously. In addition to daidzein and genistein 7-methyl ether, which presumably originate from the nutrient medium, we also isolated 2-(3-indolyl)ethanol.

The (+)- and (−)-ESI mass spectra of the compounds, termed akashins A, B, and C,[7] showed molecular weights of 475, 517, and 545 Dalton. The isotope pattern of the molecular ion indicated the presence of two chlorine atoms. The typical UV spectra, the chemical shifts of the sp^2 carbon atoms, the coupling constants of the arene protons in the ^{1}H NMR spectrum, as well as the H,H-COSY, HMQC, and HMBC correlations corroborated the structure of the aglycon (Figure 1). Furthermore, this structure is consistent with the fragment ion at *m/z* 330, which is common to all three

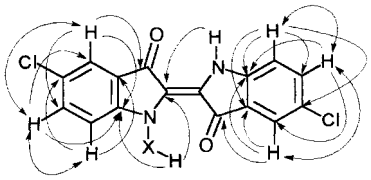


Figure 1. H,H-COSY (↔) and HMBC couplings (H →C) of the aglycon of the akashins; X = sugar residue.

pigments, and with the formula $C_{16}H_8N_2O_2Cl_2$, which was determined by means of high-resolution mass spectrometry. The akashins are derivatives of 5,5′-dichloroindigo **1b** in which the nitrogen atom of one half of the chromophore is *N*-glycosidically bound to a sugar residue, as shown by long-range coupling experiments. Surprisingly, the resulting UV/Vis absorption shows only a minor hypsochromic shift relative to that of 5,5′-dichloroindigo,[8] which was not expected based on studies of *N*,*N*′-dimethylindigo.[9]

A comparison of the coupling constants of the akashins (between $^3J = 8.6$ and 15.8 Hz) with those of bagougeramines A and B[10] ($^3J_{ax,ax} = 9.3 - 10.7$ Hz), spicamycin tetraacetate[11] ($^3J_{ax,ax} = 10.5$ Hz), and methyl 2,3-di-*O*-acetyl-4,6-dideoxy-4-(*N*,*N*′-dimethylamino)-α-D-mannopyranoside[12] ($^3J_{ax,ax} = 8.2$ Hz) reveals that all the ring protons of the sugar residue of **3a**–**3c** adopt axial positions. This is confirmed by the small axial/equatorial and equatorial/equatorial coupling constants, for example, in spicamycin tetraacetate[11] ($^3J = 3.0$ and 3.5 Hz) and methyl 4-bromo-4,6-dideoxy-α-D-talopyranoside[13] ($^3J = 1.5 - 4.1$ Hz). To confirm further the configuration of the sugar residue, the absolute energy minimum of **3a** was calculated by using the HUNTER program.[14] The coupling constants determined for the calculated conformation by using PCModel agreed well with the experimental values.

[*] Prof. Dr. H. Laatsch, Dr. R. P. Maskey
Institut für Organische Chemie
Georg-August-Universität Göttingen
Tammannstrasse 2, 37077 Göttingen (Germany)
Fax: (+49) 551-399660
E-mail: HLAATSC@gwdg.de
Dr. I. Grün-Wollny
Fa. bioLeads, Heidelberg (Germany)
Prof. Dr. H. H. Fiebig
Oncotest GmbH, Freiburg (Germany)

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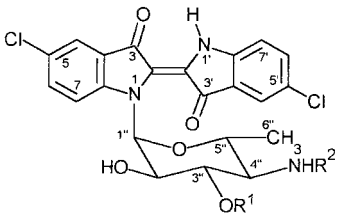
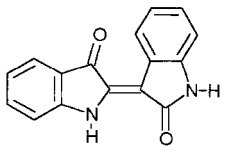
The sugar residue is 4-acetamido-4,6-dideoxyglucopyranose; the corresponding amino sugar is a component of more than 120 natural products, for example, tallysomycin,[15] amicetin,[16] calicheamicin,[17] and norplicacetin,[18] and is found in both the D and L configurations. It has thus far not been possible to determine which form occurs in the akashins, as a result of the small quantities of material obtained. Further investigations are required to determine if the extremely large optical rotation of the akashins is a result of a helical conformation of the chromophore.

Similar to indigo, 5,5′-dichloroindigo **1b** is poorly soluble in cold common organic solvents. The akashins are even less soluble than **1b** in nonpolar solvents; however, they are more soluble in methanol and dimethyl sulfoxide, but not in water.

Although indigo itself shows no biological activity, the akashins **3a**–**c** are active against various human tumor cell





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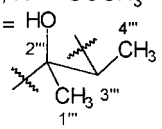
3a: $R^1 = R^2 = H$
3b: $R^1 = H$, $R^2 = COCH_3$
3c: R^1, R^2 = 

lines (CNCL SF268, LCL H460, MACL; colon carcinoma CCL HT29, melanoma MEXF 514L, lung carcinoma LXFA 526L, LXFL, and 529L, breast cancer MCF-7, kidney tumors PRCL PC3M and RXF 631L) with IC_{50} values of about 2.8 μg mL^{-1} and IC_{70} values of >3 μg mL^{-1}. Thus they resemble the 2,3-coupled indirubin (**4**), an ingredient of the Chinese drug Danggui Longhui Wan, for which strong antitumor activity was found.[19] In contrast to indigo, however, the akashins are not accompanied by red isomers.

Experimental Section

The terrestrial *Streptomyces* GW 48/1497 (bioLeads, Heidelberg) was fermented and handled under standard conditions[20] on a soya–mannitol medium (shaker culture, 15 L divided into 75 1-L baffled flasks), 3 days, 28 °C, 110 Upm).

After separation on silica gel, the blue pigment fraction was purified by means of preparative thin-layer chromatography (PTLC) ($CHCl_3$/15% MeOH/0.1% AcOH). Concentration of the eluate from band at $R_f = 0.15$ ($R_f = 0.11$, CH_2Cl_2/10% MeOH) gave **3a** (35.6 mg) as a blue substance which is only sparingly soluble in methanol. Further purification of two subsequent fractions led to the isolation of **3b** and **3c** as dark blue substances: purification on Sephadex LH-20 (3 × 70 cm, $CHCl_3$/MeOH (40%)) gave **3b** (6 mg; $R_f = 0.48$, $CHCl_3$/17% MeOH/0.2% AcOH; $R_f = 0.22$, CH_2Cl_2/10% MeOH); purification of the second fraction on Sephadex LH-20 and PTLC (20 × 20 cm, $CHCl_3$/10% MeOH) gave **3c** (5 mg; $R_f = 0.49$ ($CHCl_3$/17% MeOH/0.2% AcOH; $R_f = 0.52$ (CH_2Cl_2/10% MeOH).

Akashin A (**3a**): ^{1}H NMR ([D$_6$]DMSO, 300 MHz): $\delta = 11.01$ (br s, H/D exchangeable, 1H; N′-H), 7.75 (d, $^4J = 1.8$ Hz, 1H; 4-H), 7.65 (dd, $^3J = 8.8$ Hz, $^4J = 2.2$ Hz, 1H; 6-H) 7.60, 7.59, 7.57 (m, 3H; 4′-, 7-, 6′-H), 7.44 (dd, $^3J = 8.5$ Hz, $^5J = 0.6$ Hz, 1H, 7′-H), 5.80 (d, $^3J = 9.2$ Hz, 1H; 1″-H), 5.64 (br s, H/D exchangeable, 1H; OH), 5.11 (d, H/D exchangeable, $^3J = 7.2$ Hz, 1H; OH), 3.79 (qd, $^3J = 9.5$ Hz, $^3J = 6.1$ Hz, 1H; 5″-H), 3.62 (dd, $^3J = 15.8$ Hz, $^3J = 8.6$ Hz, 1H; 2″-H), 3.30 (dd, $^3J = 15.8$ Hz, $^3J = 9.8$ Hz, 1H; 3″-H), 2.81 (t, $^3J = 9.8$ Hz, 1H; 4″-H) 1.43 (d, $^3J = 6.0$ Hz, 3H; 6″-H$_3$); ^{13}C NMR ([D$_6$]DMSO, 125.7 MHz): $\delta = 186.4$ (CO-3), 185.3 (CO-3′), 150.6 (C-7a′), 148.7 (C-7a), 135.6 (CH-6′), 134.5 (CH-6), 126.4 (C-5), 124.7 (C-5′), 124.3 (C-2), 123.9 (C-2′), 123.8 (C-3a), 123.0 (CH-4′), 122.5 (CH-4), 120.0 (C-3a′) 117.7 (CH-7), 115.0 (CH-7′), 87.6 (CH-1″), 74 (CH-3″), 72.1 (CH-5″), 69.7 (CH-2″), 57.3 (CH-4″), 18.0 (5″-Me); (+)-ESI-MS: *m*/*z* (%): 976 (47) [2*M*+Na], 974 (87) [2*M*+Na], 972 (65) [2*M*+Na], 502 (10) [*M*+], 500 (60) [*M*+], 498 (98) [*M*+], 480 (8) [*M*+1], 478 (32) [*M*+1], 476 (48) [*M*+1], 335 (11) [*M* − sugar], 333 (65) [*M* − sugar], 331 (100) [*M* − sugar], 146 (84) [sugar]; (−)-ESI-MS: *m*/*z* (%): 478 (33) [*M* − 1], 476 (100) [*M* − 1], 474 (98) [*M* − 1]; UV/Vis (MeOH): λ_{max} (lg ε) = 241 (4.33), 290 (4.23), 619 (4.01) nm; IR (KBr): $\tilde{\nu} = 3426$, 2927, 1636 (CO), 1610, 1463, 1386, 1306, 1259, 1180, 1120, 1084, 1048, 883, 829, 764, 710, 649, 623, 585 cm^{-1}; $[\alpha]_D^{25} = +2560°$ ($c = 12.5$ μg mL^{-1}, MeOH); calcd for $C_{16}H_8N_2O_2Cl_2$ (*M* − sugar + H): 329.9962, found: 329.9962.

Akashin B (**3b**): ^{1}H NMR ([D$_6$]DMSO, 500 MHz): acetate signal at $\delta = 1.86$ (s, 3H; NHAc), other signals similar to those of **3a**; ^{13}C NMR ([D$_6$]DMSO, 125.7 MHz): $\delta = 170.0$ (NH*C*O-Me), 23.1 (NHCO-*Me*), other signals similar to those of **3a**; UV/Vis (MeOH): λ_{max} (lg ε) = 244 (4.34), 291 (4.28), 618 (4.03) nm; IR (KBr): $\tilde{\nu} = 3430$, 2929, 1641 (CO), 1610, 1558, 1464, 1384, 1305, 1259, 1182, 1087, 1053, 884, 824, 764, 709 cm^{-1}; $[\alpha]_D^{25} = +2840°$ ($c = 14$ μg mL^{-1}, MeOH).

Akashin C (**3c**): ^{1}H NMR (CD$_3$OD, 500 MHz): $\delta = 7.74$ (d, $^3J = 9.1$ Hz, H/D exchangeable, 1H; N-H), 7.70 (d, $^4J = 2.1$ Hz, 1H; 4-H), 7.58 (m, 3H; 6-H, 7-H, 4′-H), 7.49 (dd, $^3J = 8.8$ Hz, 1H; 6′-H), 7.18 (d, $^3J = 8.7$ Hz, 1H; 7′-H), 6.12 (d, $^3J = 9.2$ Hz,1H; 1″-H), 3.97 (t, $^3J = 10.1$, 1H; 2″-H), 3.95 (dq, $^3J = 9.6$ Hz, $^3J = 6.6$ Hz, 1H; 5″-H), 3.92 (t, $^3J = 9.6$ Hz, 1H; 3″-H), 2.82 (q, $^3J = 6.5$ Hz, 1H; 3‴-H), 2.70 (t, $^3J = 9.6$ Hz, 1H; 4″-H), 1.45 (d, $^3J = 6.6$ Hz, 3H; 6″-H$_3$), 1.20 (d, $^3J = 6.5$ Hz, 3H; 4‴-H$_3$), 1.18 (s, 3H; 1‴-H$_3$); ([D$_6$]DMSO): additionally $\delta = 10.98$ (s, H/D exchangeable, 1H; N′-H); ^{13}C NMR (CD$_3$OD, 125.7 MHz): $\delta = 188.8$ (CO-3), 187.4 (CO-3′), 152.0 (C-7a′), 150.5 (C-7a), 137.1 (CH-6′), 136.0 (CH-6), 129.9 (C-5), 129.0 (C-3a), 127.3 (C-5′), 126.6 (C-2′), 125.6 (C-2), 124.6 (CH-4′), 124.1 (CH-4), 122.0 (C-3a′), 118.7 (CH-7), 115.2 (CH-7′), 96.7 (C-2‴), 89.7 (CH-1″), 75.1 (CH-5″), 75.0 (CH-3″), 69.2 (CH-2″), 62.9 (CH-4″), 59.2 (CH-3‴), 25.6 (1‴-Me), 18.0 (5″-Me), 16.3 (3‴-Me); (+)-ESI-MS: *m*/*z* (%): 570 (4) [*M*+Na], 568 (16) [*M*+Na], 546 (28) [*M*+1]; (−)-ESI-MS: *m*/*z* (%): 548 (54) [*M* − 1], 546 (96) [*M* − 1], 544 (100) [*M* − 1]; UV/Vis (MeOH): λ_{max} (lg ε) = 241 (4.58), 290 (4.43), 619 (4.20) nm; IR (KBr): $\tilde{\nu} = 3447$, 1638 (C=O), 1610, 1467, 1390, 1308, 1260, 1180, 1080, 1049, 887, 829, 764, 710 cm^{-1}; $[\alpha]_D^{25} = +3100°$ ($c = 5.8$ μg mL^{-1}, MeOH).

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[20] Fat-free soya flour (20 g) and mannitol (20 g) were taken up in tap water (1 L). The pH was adjusted to 7.8 before sterilization.

Wet Chemistry Synthesis of β-Nickel Aluminide NiAl**

Helmut Bönnemann,* Werner Brijoux, Hans-Werner Hofstadt, Teyeb Ould-Ely, Wolfgang Schmidt, Bernd Waßmuth, and Claudia Weidenthaler

Owing to its extraordinary physical properties β-nickel aluminide NiAl is an important raw material,[1] which on the one hand has a lower density than nickel,[2] while on the other at 1638 °C[3] exhibits a significantly higher melting point than nickel (1453 °C) and aluminum (660 °C). Since a dense aluminum oxide layer is formed, even at low surface oxidation, NiAl has high thermal stability and resistance to atmospheric oxygen.[4] Owing to its high resistance towards environmental influences NiAl can be used as a Ni replacement, for example in gas turbine blades and automobile construction.[5]

The mechanical properties of NiAl are highly dependent upon the conditions of preparation and grain size.[6] Traditionally, the coarse-grained material ($\varnothing > 1$ μm), which is produced by milling[7] at 800 – 900 °C("mechanical alloying") is brittle at room temperature. Refining the particle diameter into the nanometer range increases ductility, strength, and hardness.[8] Thus nanocrystalline NiAl, which was produced by gas-phase condensation,[9] exhibits a considerably greater microhardness than the milled material. Mechanical alloying is associated with two significant disadvantages: 1) the particles readily agglomerate to larger units at the relatively high milling temperature, and 2) large amounts of Ni and Al remain together unalloyed.

Buhro et al.[10] have described a method for the wet chemical synthesis of NiAl by the reaction of suspended $NiCl_2$ with $LiAlH_4$ in mesitylene while refluxing under an inert atmosphere. NiAl is not formed directly during the reaction but only by subsequently heating the solid to about 550 °C. The by-products LiCl and $AlCl_3$ must be removed from the solid by sublimation at 700 – 750 °C, whereby a relatively coarse-grained material is obtained. Withers et al[11] reacted $NiCl_2$ with Al powder at 750 °C and after purification of the crude product from $AlCl_3$ by sublimation, obtained a Ni_3Al powder with a grain size in the micrometer range ($\varnothing = 1.4 – 1.8$ μm). Abe and Tsuge[12] obtained a mixture of NiAl and Ni_3Al powder by the reaction of $NiCl_2$ and $AlCl_3$ with ammonium carboxylates and subsequent heating of the resulting Ni and Al carboxylates up to 1400 °C under argon.

As early as 1955 Ziegler and co-workers described the formation of colloidal nickel in the reaction of $[Ni(acac)_2]$ (acac = acetylacetonate) with AlR_3 (R = alkyl) in solution.[13] This finding was used later by Wilke et al. to explain the „nickel effect“ in the polymerization reaction of ethene on AlR_3;[14] in this context the reactions of organonickel complexes such as $[Ni(bpy)Me_2]$ (bpy = 2,2 – -bipyridine), [Ni(cdt)] (cdt = cyclododeca-1,5,9-triene), and $[NiCl_2(PR_3)_2]$ with AlR_3 or $AlHR_2$ were also investigated. Wilke et al. concluded that in these reactions thermolabile addition compounds of nickel core complexes were formed with AlR_3 or $AlHR_2$.[14]

Repeating the Ziegler experiments in an autoclave under H_2 we obtained a black dispersion, which according to transmission electron microscopy (TEM) contained colloidal nickel [Eq. (1)] The reaction of $[Ni(acac)_2]$ with Al*i*Bu_3 gave particles with a mean diameter of $\varnothing = 2.8$ nm. After removal of the solvent and excess $AlEt_3$ in high vacuum (HV) a hard black solid remained. By hydrogenation at 80 °C under pressure (5 MPa) we obtained from this a black powder, which according to elemental analysis contained NiAl and larger amounts of an undefined, inseparable by-product [Eq. (1)].

$$[Ni(acac)_2] + 2\,AlEt_3 + H_2 \xrightarrow{\text{toluene, } 100\,^\circ C} \text{black dispersion} \xrightarrow{HV} \text{black solid} \xrightarrow{H_2,\ 80\,^\circ C} NiAl + \text{by-products} \quad (1)$$

The reaction of $[Ni(cod)_2]$ (cod = cycloocta-1,5-diene) with $AlEt_3$ at room temperature in a molar ratio of 1:1 in toluene under a H_2 pressure of 5 – 10 MPa gave a clear black-brown dispersion from which a black solid precipitates. After

[*] Prof. Dr. H. Bönnemann, Dr. W. Brijoux, H.-W. Hofstadt, Dr. T. Ould-Ely, Dr. W. Schmidt, B. Waßmuth, Dr. C. Weidenthaler
Max-Planck-Institut für Kohlenforschung
Abteilung Heterogene Katalyse
Postfach 101353, 45466 Mülheim an der Ruhr (Germany)
Fax: (+49) 208-306-2983
E-mail: boennemann@mpi-muelheim.mpg.de

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