Monatshefte für Chemie 126, 805–809 (1995)

Monatshefte für Chemie
Chemical Monthly

Total Syntheses and Antimicrobial Activities of Pyridine Alkaloids from Rubiaceae

F. Bracher* and **T. Papke**

Institut für Pharmazeutische Chemie, Technische Universität Braunschweig, D-38106 Braunschweig, Federal Republic of Germany

Summary. Pyridine alkaloids from Rubiaceae were prepared by palladium-catalyzed cross-coupling reactions of methyl 5-bromonicotinate (**6**) with various organometallic reagents. Baker's yeast reduction of the ketone **8** gave the levorotatory alcohol (*S*)-**1**. On this basis, the naturally occuring alcohol (+)−**1** was assigned to be (*R*)-configurated. The alkaloids **1** and **4** show weak antimicrobial activities.

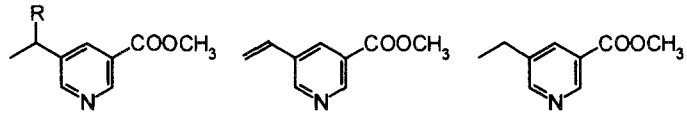
Keywords. Pyridine alkaloids; Palladium catalyst; Baker's yeast; Antimicrobial activity.

Totalsynthesen und antimikrobielle Aktivität von Pyridin-Alkaloiden aus Rubiaceen

Zusammenfassung. Pyridin-Alkaloide aus Rubiaceen wurden durch palladium-katalysierte Kupplungsreaktionen von 5-Bromnicotinsäuremethylester (**6**) mit verschiedenen organometallischen Verbindungen hergestellt. Reduktion des Ketons **8** mit Bäckerhefe ergab den linksdrehenden sekundären Alkohol (*S*)-**1**. Daher weist der natürlich vorkommende Alkohol (+)−**1** (*R*)-Konfiguration auf. Die Alkaloide **1** und **4** besitzen schwache antimikrobielle Aktivität.

Introduction

Four pyridine alkaloids (**1**–**4**) have been isolated from the root bark of the West African tree *Nauclea diderrichii* (Rubiaceae) in the early seventies [1]. Very recently, **1** has also been identified as a constituent of *Isertia haenkeana* (Rubiaceae) [2]. The 5-ethyl analogue **5** was obtained as an artefact formed from a secoiridoide glycoside from *Ligustrum vulgare* (Oleaceae) by treatment with H_2SO_4 and ammonia [3] (Scheme 1).



1 R = OH
2 R = OCH_3
3 R = NH_2

4 **5**

Scheme 1

A multistep synthetic route to the alkaloids **4** and racemic **1–3** has been developed by *McLean* and *Murray* [1]. The absolute configurations of the chiral natural products **1–3** have not yet been determined.

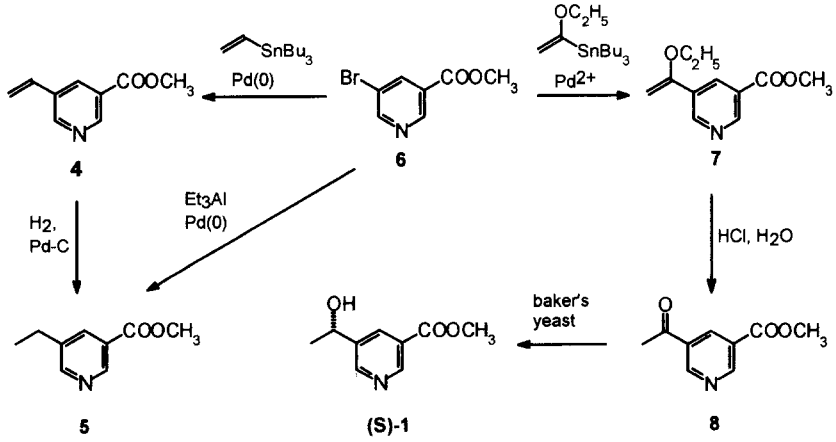
Our attention was drawn to this class of alkaloids because of the antimicrobial activity of the related marine 3-alkyl pyridines niphatesine C and ikimine A synthesized by us [4]. Thus, we worked out an efficient approach to the pyridines described above to provide sufficient amounts for an antimicrobial screening.

Results and Discussion

We selected modern transition metal-catalyzed cross-coupling reactions of bromopyridine **6** with appropriate organometallic reagents as the key steps in the syntheses of the target compounds. Methyl 5-bromopyridine-3-carboxylate (**6**) [5], readily prepared from the commercially available carboxylic acid by esterification with diazomethane, was selected as the starting material.

Pd(0)-catalyzed cross-coupling of **6** with vinyl tri-*n*-butylstannane [6] gave the vinyl compound **4** in 92% yield. Catalytic hydrogenation of **4** smoothly yielded the ethyl derivative **5**. In an even shorter approach, **5** could be prepared by Pd(0)-catalyzed ethylation of **6** with various organometallic reagents. Of the three reagents triethylaluminum, triethylborane, and diethylzinc [7] examined, the aluminum compound gave the best yield (24%). Triethylborane afforded 17%, whereas with diethylzinc only traces of **5** were formed. In contrast, in the pyrazine series triethylborane had been shown to be superior to the other reagents [7]. In all cases reductive debromination to methyl nicotinate was a severe competing process. This undesired reaction may be caused by thermal cleavage of the organometallic reagents into ethene and metal hydrides with reducing abilities [7].

For the preparation of **1**, the methyl ketone **8** was selected as the precursor. Previously described syntheses of **8** involve multiple steps and give poor overall yields [1, 8]. We could prepare **8** in 63% yield by Pd^{2+}-catalyzed cross-coupling of



Scheme 2

6 with 1-ethoxyvinyl tributylstannane [9] and subsequent hydrolysis of the intermediate enol ether **7** with conc. HCl/methanol at room temperature. Under these conditions undesired cleavage of the methyl ester was avoided. Reduction of the ketone **8** with $NaBH_4$ is known to give racemic alcohol *rac*-**1** which can be converted to *rac*-**2** by methanolysis of its mesilate [1]. Reductive amination of **8** has been described to give *rac*-**3** [1].

For the preparation of non-racemic **1**, we used reduction of the ketone **8** by baker's yeast (*Saccharomyces cerevisiae*), since an analogous yeast-mediated reduction of 3-acetylpyridine has been described to proceed very well [10]. In fact, **8** was readily converted to the levorotatory secondary alcohol (−)-**1** by fermenting baker's yeast. The enantiomeric excesses obtained in several batches were in the range of 84–95%. The optical purity was determined by GLC analysis after derivatization with (*R*)-1-phenylethylisocyanate.

In an alternative approach, **8** was reduced by (*R*)-Alpine Borane™ (B-3-pinanyl-9-borabicyclo[3.3.1]nonane derived from (+)-α-pinene [11]). This procedure also gave levorotatory **1**. The enantiomeric excess, however, was only 26%. According to the proposed reaction mechanism of (*R*)-Alpine Borane™ [11], working under higher pressure may give better optical yields. The absolute configuration of the reduction products was determined to be (*S*) by the method described by *Dale* and *Mosher* [12]. Thus, the secondary alcohol was converted to the (*S*)-*MTPA* ester (*MTPA* = α-methoxy-α-trifluoromethylphenylacetate). Whereas in a ^{1}H NMR experiment the (*S*)-*MTPA* derivative of *rac*-**1** gave two well resolved doublets at 1.63 and 1.68 ppm for the methyl groups of the two diastereomers, in the enantiomerically enriched reduction products described above the upfield doublet at 1.63 ppm was the major one. Consequently, the major diastereomer was assigned (*S,S*)-configuration. The proposed (*S*)-configuration is consistent with the mechanism of the (*R*)-Alpine Borane™ reduction of aryl methyl ketones [11] and the known stereochemical outcome of the yeast-mediated reduction of the related 3-acetyl pyridine [10]. Consequently, the dextrorotatory naturally occuring **1** has (*R*)-configuration.

In a first screening, ketone **8** and methyl ether **2** exhibited no antimicrobial activity. Alcohol **1** and vinyl compound **4** showed weak activity against gram-positive bacteria and fungi in an agar diffusion assay.

Experimental

NMR spectra: Bruker AM 400, internal standard: *TMS*; mass spectra: Finnigan MAT 8430; IR spectra: Pye-Unicam PU-9800; analytical data (C, H, N): C–H–N–O Elemental Analyzer 1106, Carlo Erba; GLC: Shimadzu GC-14 A equipped with FID; flash column chromatography: Kieselgel 60 (230–400 mesh), Merck.

Methyl 5-bromonicotinate (**6**)

To an ice-cooled suspension of 5-bromo nicotinic acid (1.6 g, 7.9 mmol) in diethyl ether (20 ml), a solution of diazomethane in diethyl ether was added dropwise with stirring until the mixture remained yellow. The mixture was stirred for additional 15 min (hood!), and the volatile compounds were removed by distillation *in vacuo*. The residue **6** was pure enough to be used in the next step without further purification. The spectroscopic data of **6** were in accordance with the values published in the literature [5].

Methyl 5-vinylnicotinate (**4**)

To a solution of **6** (216 mg, 1.0 mmol) in anhydrous toluene (2 ml), a few crystals of 2,6-di-*tert.*-butyl-4-methylphenol, $Pd(Ph_3P)_4$ (46 mg, 0.04 mmol) and vinyl tri-*n*-butylstannane (475 mg, 1.5 mmol) were added under N_2. The mixture was refluxed for 3 h. After cooling, the residue (Pd) was filtered off and the filtrate was treated with ethyl acetate (10 ml), water (20 ml), and saturated aqueous KF-solution. The organic layer was separated, dried and concentrated *in vacuo*. The residue was recrystallized from ice-cooled hexane to give 150 mg (92%) **4** as colourless needles. M.p.: 50–60 °C (Ref. [1]: 50–75 °C); IR (KBr): $\nu = 3005$, 1728, 1285, 1211, 1111, 770 cm^{-1}; ^1H NMR ($CDCl_3$): $\delta = 9.09$ (d, $J = 2.0$ Hz, 1H, 2-H), 8.77 (d, $J = 2.2$ Hz, 1H, 6-H), 8.33 (dd, $J = 2.0$ Hz, $J = 2.2$ Hz, 1H, 4-H), 6.75 (dd, $J = 11.0$ Hz, $J = 17.6$ Hz, 1H, 1'-H), 5.93 (d, $J = 17.6$ Hz, 1H, 2'-H), 5.48 (d, $J = 11.0$ Hz, 1H, 2'-H), 3.97 (s, 3H, OCH_3) ppm; MS (70 eV): *m/z* (%) = 163 (82) [M^+], 132 (100), 104 (85), 77 (43); $C_9H_9NO_2$ (163); calcd.: 163.0633, found: 163.0633.

Methyl 5-ethylnicotinate (**5**)

Method A: To a solution of **4** (30 mg, 0.18 mmol) in ethanol (2 ml), palladium on charcoal (5% Pd; 15 mg) was added and the mixture was hydrogenated at room temperature for 1 h. Then the catalyst was removed by filtration and the solvent was removed *in vacuo* to give 20 mg (66%) **5** as a colourless oil. IR (film): $\nu = 2967$, 1728, 1291, 1213, 1111 cm^{-1}; ^1H NMR ($CDCl_3$): $\delta = 9.05$ (d, $J = 2.0$ Hz, 1H, 2-H), 8.62 (d, $J = 2.2$ Hz, 1H, 6-H), 8.13 (dd, $J = 2.0$ Hz, $J = 2.2$ Hz, 1H, 4-H), 3.95 (s, 3H, OCH_3), 2.72 (q, $J = 7.4$ Hz, 2H, CH_2), 1.29 (t, $J = 7.4$ Hz, 3H, CH_3); MS (70 eV): *m/z* (%) = 164 (80) [M^+], 134 (100), 106 (55); $C_9H_{11}NO_2$ (165); calcd.: C 65.44, H 6.71, N 8.48; found: C 64.75, H 6.90, N 8.36.

Method B: To a solution of **6** (216 mg, 1.0 mmol) in anhydrous dioxane (4 ml) under N_2, $Pd(Ph_3P)_4$ (23 mg, 0.02 mmol) and triethylaluminum (1 *M* in hexane; 1.5 ml, 1.5 mmol) was added and the mixture was refluxed for 90 min. After cooling, water (10 ml) was added followed by extraction with ethyl acetate. The organic layer was separated, dried, and concentrated *in vacuo*. The residue was purified by flash column chromatography to give 40 mg (24%) of **5**.

Methyl 5-acetylnicotinate (**8**)

A solution of **6** (1.7 g, 7.9 mmol), $PdCl_2(Ph_3P)_2$ (280 mg, 0.4 mmol), and 1-ethoxyvinyl tri-*n*-butylstannane (3.61 g, 10.0 mmol) under N_2 was refluxed for 3 h. After cooling, the mixture was filtered and the volatile compounds were evaporated *in vacuo*. The oily residue was dissolved in methanol (8 ml), and 10 *M* HCl (8 ml) was added. The mixture was stirred at room temperature for 2 h and then diluted with water (20 ml) and made alkaline by addition of sodium carbonate. Extraction with ethyl acetate (3 × 50 ml), drying of the organic layer and evaporation *in vacuo* gave the crude product. Purification by flash column chromatography afforded 0.89 g (63%) **8** as pale yellow needles. M.p.: 88–91 °C (Ref. [1]: 90–92 °C); IR (KBr): $\nu = 1719$, 1682, 1595, 1256, 1237 cm^{-1}; ^1H NMR ($CDCl_3$): $\delta = 9.37$ (d, $J = 2.0$ Hz, 1H, 2-H), 9.32 (d, $J = 2.2$ Hz, 1H, 6-H), 8.80 (dd, $J = 2.0$ Hz, $J = 2.2$ Hz, 1H, 4-H), 4.00 (s, 3H, OCH_3), 2.70 (s, 3H, CH_3); MS (70 eV): *m/z* (%) = 179 (41) [M^+], 164 (100), 148 (16), 136 (40); $C_9H_9NO_3$ (179); calcd.: C 60.33, H 5.06, N 7.82; found: C 60.17, H 5.06, N 7.60.

(S)-Methyl 5-(1'-hydroxyethyl)-nicotinate ((*S*)-**1**)

A mixture of commercial baker's yeast (*Dr. Moormann's Beste*; 42 g), glucose (20 g), $MgSO_4$ (40 mg), $(NH_4)_2HPO_4$ (60 mg), and tap water in a round bottom flask was placed in a shaking water bath and incubated at 400 rpm for 2 h at 30 °C. Then a solution of **8** (300 mg, 1.68 mmol) in ethanol (3 ml) was added. After 24 h of fermentation, the mixture was submitted to continuous extraction with ethyl acetate for 24 h. The organic layer was dried and concentrated *in vacuo*. The residue was purified by

flash column chromatography to give 203 mg (67%) (*S*)-**1** as a colourless oil. $[\alpha]_D^{20} = -25.7°$ ($c = 3.5$, $CHCl_3$; ee = 95%); IR (film): $\nu = 3314, 1728, 1429, 1294, 1239, 1211, 1111, 768\,cm^{-1}$; ^1H NMR ($CDCl_3$): $\delta = 9.06$ (d, $J = 1.8$ Hz, 1H, 2-H), 8.72 (d, $J = 2.2$ Hz, 1H, 6-H), 8.33 (dd, $J = 1.8$ Hz, $J = 2.2$ Hz, 1H, 4-H), 5.02 (q, $J = 6.5$ Hz, 1H, 1'-H), 3.2 (br. s, 1H, OH), 3.96 (s, 3H, OCH_3), 1.55 (d, $J = 6.5$ Hz, 3H, CH_3); MS (70 eV): m/z (%) = 181 (25) [M^+], 166 (100), 138 (44), 134 (26), 106 (18); $C_9H_{11}NO_3$ (181); calcd.: C 59.66, H 6.12, N 7.73; found: C 58.99, H 6.45, N 7.21.

Determination of enantiomeric excess: Alcohol **1** (50 µl) was added to a solution of (*R*)-phenylethylisocyanate (25 µl) in anhydrous dichloromethane (100 µl) in a *Wheaton* vial. The mixture was heated at 100 °C for 3 h and, after cooling, diluted with dichloromethane (3 ml). GLC analysis (AT-50 column, 265 °C isothermal, carrier: H_2) gave two peaks with $t_R = 4.76$ min (major peak; derivative of (*S*)-**1**) and $t_R = 4.90$ min (derivative of (*R*)-**1**).

Acknowledgement

This work was supported by the *Fonds der Chemischen Industrie*. We thank Dr. *W. F. Fleck, Hans-Knöll-Institut für Naturstoff-Forschung, Jena*, for performing the screening experiments for antimicrobial activity.

References

[1] McLean S, Murray DG (1971) Can J Chem **50**: 1478
[2] Bruix M, Rumbero A, Vazquez P (1993) Phytochemistry **33**: 1257
[3] Willems M (1987) Arch Pharm (Weinheim) **320**: 1245
[4] Bracher F, Papke T (1994) Nat Prod Lett **4**: 223
[5] Thompson WJ, Gaudino J (1984) J Org Chem **49**: 5237
[6] McKean DR, Parrinello G, Renaldo AF, Stille JK (1987) J Org Chem **52**: 442
[7] Ohta A, Ohta M, Igarashi Y, Saeki K, Yuasa K, Mori T (1987) Heterocycles **26**: 2449
[8] Chowdhury US (1990) Tetrahedron **46**: 7893
[9] Kosugi M, Sumiya T, Obara Y, Suzuki M, Sano H, Migita T (1987) Bull Chem Soc Jpn **60**: 767
[10] Takeshita M, Terada K, Akutsu N, Yoshida S, Sato T (1987) Heterocycles **26**: 3051
[11] Midland MM, McLoughlin JI (1984) J Org Chem **49**: 1316
[12] Dale JA, Mosher HS (1973) J Am Chem Soc **95**: 512

Received January 5, 1995. Accepted January 20, 1995