Microbial Hydroxylations of β-Carboline Derivatives

Günter Neef,* Ulrich Eder, Karl Petzoldt, Arne Seeger, and Heinz Wieglepp

Research Laboratories of Schering AG Berlin/Bergkamen, D-1000 Berlin 65, West Germany

Ethyl β-carboline-3-carboxylate (**1a**) and its 4-alkyl derivatives (**1b—d**) are hydroxylated by *Sporotrichum sulfurescens* at C-6 and C-8 of the aromatic nucleus; side chain hydroxylation of (**1c**) and (**1d**) occurs with *Streptomyces lavendulae* and *Streptomyces griseus*.

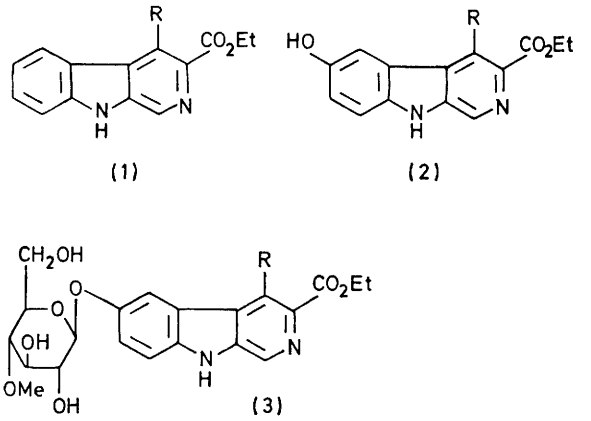
Recently, ethyl β-carboline-3-carboxylate (**1a**) was isolated from human urine by Braestrup and co-workers, who demonstrated that (**1a**) possessed high affinity for benzodiazepine-binding proteins.[1] Although it remains doubtful whether (**1a**) represents an endogenous ligand of the benzodiazepine receptor,[2] chemical interest in β-carboline derivatives has been restimulated by Braestrup's observations.

Microbial functionalization in this alkaloid class had not been investigated; we, therefore, started a screening programme involving 92 commonly used micro-organisms of which three fungi were found to effect preparatively useful conversions of (**1a**) and some 4-alkyl substituted derivatives of (**1a**): *Sporotrichum sulfurescens* ATCC 7195, *Streptomyces lavendulae* ATCC 8664, and *Streptomyces griseus* ATCC 10 137.

Sporotrichum sulfurescens ATCC 7195, although hitherto uncommon in aromatic hydroxylation,[3] turned out to be the most useful species. Fermentation of (**1a**) with ATCC 7195, using standard procedures,[4] resulted in the formation of the 6-hydroxy-derivative (**2a**) accompanied by small amounts of the glucosides (**3a**) and (**4a**) (Figure 1).† The unusual formation of 4′-*O*-methyl-β-glucosides is a characteristic feature of ATCC 7195, as previously mentioned by Kieslich *et al.*[4]

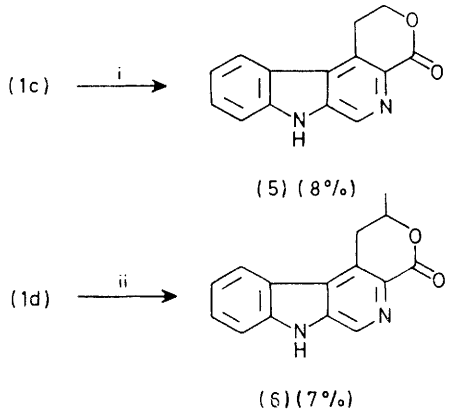
The introduction of 4-alkyl substituents into the carboline skeleton essentially influenced the regioselectivity of microbial attack. Whereas (**1a**) was predominantly affected at C-6, compound (**1b**) was converted into an almost equal mixture of 6- and 8-hydroxylated derivatives, isolated as their 4′-*O*-methyl-β-glucosides (**3b**) and (**4b**); functionalization at C-6 was completely suppressed with compounds (**1c**) and (**1d**), which, in good yields, were transformed into their 8-hydroxy-derivatives, again isolated as the glucosides (**4c**) and (**4d**).

Streptomyces lavendulae ATCC 8664 and *Streptomyces griseus* ATCC 10 137 are capable of hydroxylating the side



Reactant	6-hydroxylation	6-glucoside	8-glucoside
(**1a**)	62%	8%	7%
(**1b**)	—	20%	18%
(**1c**)	—	—	70%
(**1d**)	—	—	68%

Figure 1



i, *Streptomyces lavendulae*; ii, *Streptomyces griseus*.

† Structural assignments were made on the basis of ^{1}H n.m.r., i.r., and u.v. spectra as well as elemental analysis.
(**2a**): m.p. 248—250 °C; ^{1}H n.m.r. ([$^2H_6$]Me_2SO) δ 1.40 (3H, t, *J* 7 Hz, CO_2CH_2*Me*), 4.42 (2H, q, *J* 7 Hz, CO_2*CH*$_2$Me), 7.18 (1H, dd, *J* 9 and 2 Hz, H-7), 7.56 (1H, d, *J* 9 Hz, H-8), 7.70 (1H, d, *J* 2 Hz, H-5), 8.86 (1H, s, H-4), 8.93 (1H, s, H-1), 9.36 (1H, s, 6-OH), and 11.88 (1H, s, NH).
(**3a**): m.p. 270—272 °C; ^{1}H n.m.r. ([$^2H_6$]Me_2SO) δ 1.40 (3H, t, *J* 7 Hz, CO_2CH_2*Me*), 3.01—3.84 (6H, m, H-2′, H-3′, H-4′, H-5′, and H-6′), 3.52 (3H, s, 4′-OMe), 4.42 (2H, q, *J* 7 Hz, CO_2*CH*$_2$Me), 4.77 (1H, t, *J* 5 Hz, 6′-OH), 5.04 (1H, d, *J* 7.5 Hz, H-1′), 5.30 (1H, d, *J* 4.5 Hz, 3′-OH), 5.46 (1H, d, *J* 4.5 Hz, 2′-OH), 7.38 (1H, dd, *J* 9 and 2 Hz, H-7), 7.64 (1H, d, *J* 9 Hz, H-8), 8.10 (1H, d, *J* 2 Hz, H-5), 8.91 (1H, s, H-4), 8.98 (1H, s, H-1), and 11.96 (1H, s, NH).
(**4a**): m.p. 263—265 °C; ^{1}H n.m.r. ([$^2H_6$]Me_2SO) δ 1.40 (3H, t, *J* 7 Hz, CO_2CH_2*Me*), 3.04—3.73 (6H, m, H-2′, H-3′, H-4′, H-5′, and H-6′), 4.41 (2H, q, *J* 7 Hz, CO_2*CH*$_2$Me), 4.90 (1H, m, 6′-OH), 5.05 (d, 1H, *J* 7.5 Hz, H-1′), 5.38 (2H, m, 2′-OH and 3′-OH), 7.25 (1H, t, *J* 7.8 Hz, H-6), 7.44 (1H, dd, *J* 7.8 and 1.5 Hz, H-7), 8.09 (1H, dd, *J* 7.8 and 1.5 Hz, H-5), 8.92 (1H, s, H-4), 9.02 (1H, s, H-1), and 11.88 (1H, s, NH).

chains of (**1c**) and (**1d**) to form, with concomitant ester saponification, the lactones (**5**) and (**6**), respectively.‡ Although these transformations proceeded in poor yields, most of the starting material was recovered unchanged from the cultures and could be recycled.

Received, 21st December 1981; Com. 1444

‡ Formation of the lactone (**6**) (m.p. 325—328 °C) is a highly enantioselective process, the absolute configuration of (**6**), however, is undetermined. C.d. spectrum (Me_2SO) λ 274 ($\Delta\epsilon$ −9.27), 308 (−0.881), 338 (+0.878), and 349 nm (+1.18).

References

1 C. Braestrup, M. Nielsen, and C. E. Olson, *Proc. Natl. Acad. Sci. USA*, 1980, **77**, 2288.
2 S. S. Tenen and J. D. Hirsch, *Nature*, 1980, **288**, 609.
3 K. Kieslich, 'Microbial Transformations of Non-Steroid Cyclic Compounds,' Thieme, Stuttgart, 1976.
4 K. Kieslich, H.-J. Vidic, K. Petzoldt, and G. A. Hoyer, *Chem. Ber.*, 1976, **109**, 2259.