184. 'One-Pot' Synthesis of Raumacline from Ajmaline

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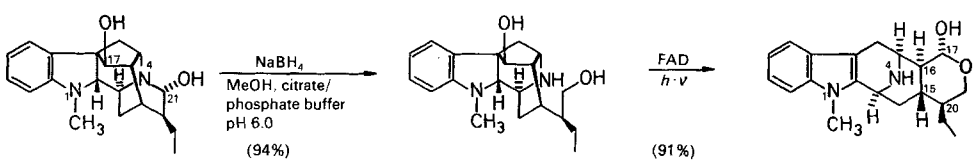
For the alkaloid raumacline (**2**), which is a biotransformation product of ajmaline (**1**) in *Rauwolfia serpentina* cell cultures, an efficient 'one-pot' synthesis was developed using a $NaBH_4$/riboflavin/light-mediated transformation of **1** into **2** with a total yield of 86%.

Introduction. – Cell-suspension cultures of *Rauwolfia serpentina* (L.) BENTH. were recently shown to efficiently transform the *Rauwolfia* alkaloid ajmaline (= (17*R*,21*R*)-ajmalan-17,21-diol; **1**), which was added in concentrations of up to 1 g l^{-1} nutrition medium [1]. Detailed analysis of the alkaloid pattern of such ajmaline-treated cells revealed a total of eight alkaloids. Six of them represented a novel alkaloid group which we named raumaclines [2] [3]. Under optimized conditions the major biotransformation product is raumacline (= (16α,17α,20β)-4-demethyl-20,21-dihydroalstophyllan-17-ol; **2**) which is formed in a yield of *ca.* 9% of the supplied ajmaline (**1**). In contrast to this relatively low transformation rate, a simple chemical synthesis of **2** was described starting with the reduction of **1** followed by N(4) protection with (benzyloxy)carbonyl chloride, oxidation of the resulting carbamate with lead tetraacetate, and removal of the N(4)-protecting group [1].

Scheme



1 ajmaline — **3** 4,21-secoajmaline — **2** raumacline

To elucidate the mechanism of the *in vivo* formation of **2** from **1** we performed a series of enzymatic experiments using cell-free extracts from cultivated *Rauwolfia* cells. Although we could not demonstrate the enzyme-catalyzed formation of **2**, we did observe a riboflavin/light-mediated transformation of 4,21-secoajmaline (= (17*R*)-4,21-secoajmalan-17,21-diol; **3**) into **2**. Based on this finding, we developed an efficient 'one-pot' synthesis of **2**, which is described in this paper.

Results and Discussion. – As we previously reported, cultivated cell suspensions of the Indian medicinal plant *Rauwolfia serpentina* are not solely a rich source of known and novel alkaloids [4]. They also can be successfully employed in the isolation of a whole

series of highly specific enzymes involved in the biosynthesis of *Rauwolfia* alkaloids [4] and proteins catalyzing common reactions, *e.g.* glucosylations [5]. Moreover, these cells exhibit, under certain conditions, exceptionally high *in vivo* biotransformation capabilities [1] [6]. From these more recent results, it seems that cultivated plant cells are able to perform very high-yielding and unexpected reactions, one being the transformation of the typical *Rauwolfia* alkaloid ajmaline (**1**) into the novel raumacline alkaloids. Based on this biotransformation process, a four-step synthesis of the raumacline skeleton and of raumacline (**2**) itself was developed [1]. So far, however, all our effort in searching for soluble enzymes responsible for the ajmaline transformation has failed. Because the raumaclines are obviously formed by the cell culture from the exogenous added **1**, rather than from the endogenous biosynthesized alkaloid, one cannot exclude that membrane- or cell-wall-bound proteins participate in this process. Again, all our cell-free experiments with membrane and cell-wall preparations were negative, when **1** was used as a substrate. In the instances when FAD or FMN[1]) was present in the incubation mixture together with 4,21-secoajmaline (**3**), we could detect nearly complete conversion into **2** (data not shown). Its formation could also be monitored in the presence of heat-denatured proteins, pointing clearly to a spontaneous reaction which was not enzyme-catalyzed.

The rate of raumacline synthesis was difficult to reproduce in these incubation mixtures and increased with light. From these observations, we were able by HPLC monitoring to optimize the reaction to a yield of > 90% by irradiating a solution of **3** in MeOH and citrate/phosphate buffer with a 1000-W halogen lamp. When the reaction dependence of FAD was determined, it was found that the oxidizing agent could be applied in catalytic amounts with 0.5 mmol as a concentration allowing the transformation of 3.07 mmol (1 g) of **3** to completion in an acceptable irradiation time of 22 h. Moreover, FAD could simply be replaced by the much cheaper riboflavin resulting in 92% transformation after only 8 h of irradiation. Because **3** was easily obtained in more than 94% yield by reduction with $NaBH_4$ of ajmaline (**1**) in the above mentioned buffer system, the complete synthesis of **2** starting with **1** could be performed in a 'one-pot' procedure with a yield of 86%, making now **2** available in a higher gram-range for biological testing.

In preliminary experiments, we also demonstrated that the flavin/light-mediated reaction can be applied not only to a variety of alkaloids of the indoline (= 2,3-dihydro-1*H*-indole)-type, but also to other structural types leading to interesting transformation products in remarkable yields [7], far exceeding those usually obtained by $Pb(OAc)_4$ oxidation [8–10]. The here described raumacline synthesis can, therefore, be seen as an attractive alternative to the $Pb(OAc)_4$ oxidation, making use of the photochemical properties of the flavins.

Flavin-sensitized photooxidations are known for a wide range of substrates. *E.g.*, the reaction of amino acids in the presence of flavins [11] [12] or the photodynamic action with drugs like phenothiazines [13] or antibiotics [14] were studied. Although the photoactivity of the flavins is well known for a long time, they were, to our knowledge, up to now only rarely used as reagents in alkaloid synthesis [15]. The predominant part of the publications deals with the undesired degradation of substrates due to the photoreaction or with the mechanism of these reactions.

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[1]) Abbreviations: FAD: Flavin-adenine dinucleotide, FMN: flavin mononucleotide.

Experimental Part

Chromatographic Methods. TLC: 0.2-mm silica-gel plates F_{254} (*Merck*, Darmstadt); solvent systems: AcOEt/MeOH/H_2O/25% NH_4OH 7:2:1:0.02 (*A*), $CHCl_3$/hexane/Et_2NH 2:2:1 (*B*); R_f (**2**): 0.34 (*A*) or 0.52 (*B*). Prep. TLC: 0.5-mm silica gel *60* F_{254} plates (*Merck*, Darmstadt), solvent system *A*. HPLC: *Merck-Hitachi* system (*L-6200A* pump, *L-4250* UV/VIS detector, *AS-2000* autosampler, *D–2500* chromato-integrator) coupled with a *Lichrospher 60 RP select B* column (125 × 4 mm or 250 × 4 mm); flow rate 1 ml/min; UV detection at 284 nm; solvent system: mixtures of MeCN (solvent *1*) and potassium phosphate buffer (6.66 g KH_2PO_4 and 4.8 g 85% H_3PO_4 per l; solvent *2*); short column: solvent *1*/solvent *2* 20:80→35:65 (within 5 min)→50:50 (within 3 min), t_R 4.9 (**1**), 5.3 (**3**), and 7.6 min (**2**); 250-mm column: solvent *1*/solvent *2* 10:90→45:55 (within 30 min), t_R 17.1 (**1**), 18.4 (**3**), and 23.2 min (**2**).

Raumacline (**2**). Ajmaline (1 g, 3.07 mmol; **1**) was dissolved in MeOH (100 ml) and diluted with 0.1M citrate/phosphate buffer (pH 6.0; 100 ml). The final pH of this mixture was adjusted to pH 6.0 with HCl. Monitoring the pH at 6.0, $NaBH_4$ (3.1 g, 82 mmol) was added gradually during 15 h at 4°. HPLC (external standard): 94% reduction of **1** to **3**. The excess of $NaBH_4$ was decomposed by addition of acetone (25 ml). To this mixture, a soln. of FAD (446 mg, 0.5 mmol) in citrate/phosphate buffer (20 ml) or a sat. soln. of riboflavin in the same buffer (100 ml, pH 6.0, containing 14 mg (37.2 μmol) of riboflavin) was added. The mixture was then placed in a 1.5-l *Fernbach* flask and stirred for 22 or 8 h, resp., at 4° under continuous irradiation with a 1000-W halogen lamp (type tungsten) from a distance of 80 cm (22000 lux). HPLC: 92% transformation of **3** to **2**, *i.e.* 86% overall yield for **2** from **1**. The mixture was brought to pH 9.2 by adding KOH. Extraction with 3 portions of CH_2Cl_2 (75 ml) and evaporation of the combined org. phase gave 960 mg of a crude extract which was purified by prep. TLC silica gel, *A*): pure **2** (564 mg; purity 99% by HPLC). IR, UV, EI-MS, ^{1}H- and ^{13}C-NMR, TLC and HPLC properties: all identical to those of previously isolated **2** from biotransformation experiments [1] and chemical synthesis [1]. $[\alpha]_D^{20} = -26$ ($c = 0.1$, $CHCl_3$) (chemical synthesis: $[\alpha]_D^{20} = -22$ ($c = 0.1$, $CHCl_3$)).

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