Concept and Development of a Potent Topical Corticosteroid [1][2]

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Abstract. In a rational approach to identify an ultrapotent compound for the treatment of therapy-resistant dermatoses, Weirich's modification [8] of McKenzie's skin vasoconstriction assay (HVK test) has been used as the essential selection criteria. In a primary phase, a quantitative relationship between the HVK activity of 25 derivatives of corticosterone substituted in various positions of the skeleton, and their lipophilicity (log P) was established. The specific lipophilicity-independent interactions were accounted for by the inclusion of 'indicator variables' into the regression analysis. The highly significant results allowed to localize an optimal log P range and to identify the influence of various substituents. In a next phase, the evidence of the first HVK analysis was refined by considering 28 additional compounds. On the basis of the confirmed facts, six 21-chloro-6α-fluoro compounds were specifically synthesized and submitted to dermatopharmacological testing. Finally, CGP 14458 (= 21-chloro-6α,9-difluoro-1β,16β-hydroxy-16β-methyl-3,20-dioxopregna-1,4-dien-17α-yl propionate) which was predicted to be the most potent representative of these series, whose synthesis is described in detail, showed indeed to be the most effective compound. Clinical trials with this compound - halobetasol propionate/Ultravate® (ulobetasol/Miracorten®) - confirmed its unique efficacy, especially in the treatment of severe, chronic plaque psoriasis.

General

The corticosteroid hormones produced by the adrenals, as well as their more potent synthetic analogues, are representing a type of compounds of vital importance to men, showing a very broad spectrum of activity.

During the past decades, beside the classical systemic application of glucocorticosteroids, which, in spite of the great popularity of NSAID's, is still considered to be the essential treatment of many life-threatening diseases, there have been introduced into therapy the so-called dermatocorticoids (DC). Their use for the topical treatment of skin allergies, inflammations, and proliferating dermatoses of the human skin has become indispensable.

Some fifteen years ago, we have decided to try to use a rational [9] approach to the development of a 'superstrong' DC for an effective treatment of obstinate dermatoses which do not respond to the treatment with strong compounds, e.g. betamethasone valerate.

Besides the classical antiinflammatory tests, like e.g. Tonelli's 'rat-ear-dermatitis-inhibition test' [10], a high activity of the test compounds in one of the most relevant dermato-pharmacological assays - the McKenzie's 'human-skin vasoconstriction test' [11] - in a version developed in our laboratories (the HVK test) [8] was chosen for the characterization of DC's as the primary selection criteria.

It has been shown in many reports about percutaneous resorption that the flux, I, of a compound completely dissolved in a vehicle, through the skin is strongly dependent on its lipophilicity. According to Scheuplein [12], this fact can be mathematically expressed by the following equation:

$$I = \frac{dQ/dt = \frac{Pm \cdot Dm \cdot (C_s)}{d}}{d}$$

Pm = partition coefficient stratum corneum/vehicle
Dm = diffusion coefficient in stratum corneum
Δ(Cs) = difference in concentration through the stratum corneum
d = diameter of the stratum corneum

Series: HYDROCORTISONE

Series: DEXA-/FLUMETHASONE

Series: TRIAMCINOLONE / FLUCINOLONE

Series: BETAMETHASONE

Q, Y, Z = H, Hal; X = Cl, OAc, OH; R, R₁, R₂ = H, Ac; R₁+R₂ = C(CH₃)₃

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In a steady state, the amount of the compound transported per time unit through the skin is proportional to the partition coefficient between the vehicle and the stratum corneum.

We have, therefore, decided to try to deduce in the first part of our study a quantitative correlation between the HVK activity of the test compounds and their lipophilicity expressed as partition coefficients in the system octanol/H₂O, which is believed to mimic the in vivo situation best.

For the first step of our investigation, we have selected a series of 25 steroids belonging to four different groups of DC's, namely, those of hydrocortisone (HC), dexamethasone/flumethasone, triamcinolone/flucinolone and betamethasone. The lipophilicity has been manipulated mainly by variation of the substituents in positions 2, 9, and 21 of the steroid nucleus.

In (Figs. 1-4), the logarithms of the relative potencies, log \( W_{rel} \) are plotted against the log \( P \) values of the compounds. In spite of the relative large deviations of the single points from the parabola calculated by multiple regression, a clear-cut dependence is observed between the vasoconstrictor activity and the log \( P \) value. In addition, a presumably optimal log \( P \) area can be observed in the range of about 3.5-4.5.

The next step consisted in a refinement of the regression by inclusion of indicator variables in the so-called mixed Hansch-Free-Wilson approach [13]. Indicator variables, \( X_{ij} \), are discrete variables which can assume the value of either 0 or 1, describing thereby the presence or absence of a specific substituent. The corresponding coefficients, \( A_{ij} \), calculated by multiple linear regression express the contributions of the particular substituents to the overall activity. By admixing the log \( P \), a separation can be achieved between the lipophilicity-dependent contributions and specific interactions of the substituents at the molecular level. In our specific analysis, it has been shown that only the 6\( \alpha \)-fluorine, the 16\( \beta \)-Me group, and the 16,17-acetonide grouping give statistically significant contributions and have, therefore, to be considered.

As is shown in Fig. 2, the standard deviation, after introducing the above discussed corrections, is clearly in the region of the reproducibility of the biological data. This very positive result, combined with the relatively strict additivity of the log \( P \) contributions of the individual substituents on the steroid skeleton, and, therefore, with the calculability of the integral log \( P \) value of the compound, allowed us in the second series of our experiments to synthesize new derivatives, whose vasoconstrictor activity would be expected to be distinctly superior to that of compounds which we have already tested. Of special interest appeared to be the fact that a 6\( \alpha \)-F substituent having a strong positive effect upon the activity, showed no measurable contribution to the lipophilicity.

In the second analysis, there have been included 28 additional compounds. As mentioned before, particular attention has been paid to the 6\( \alpha \)-F compounds.

The relative potencies, log \( W_{rel} \) shown in Fig. 3 as a function of log \( P \), are situated in the expected range. The points carrying a CGP number correspond to six intensely synthesized compounds and to the most active DC known up today - to clobetasol propionate (CGP 9'555), the active ingredient of Glaxo's Dermovate®.

The inclusion of indicator variables leads to the shown equation (Fig. 4) [14].

The correlation having an \( r \) value of 0.98 and a standard deviation of \( s = 0.29 \) can be considered as highly satisfactory.

Below, there are reproduced the structures of the six already mentioned compounds showing a particularly high activity in the HVK test and differing in respect to their configuration at C(16), the substitution at C(2) and C(9) and in the number of C=C bonds.

They were subjected to an extended secondary screening and compared with each other in respect to activity and side
effects. A synopsis of the results obtained in the 4 most important dermatopharmacological tests [15] is presented in Fig. 5.

The UV-dermatitis inhibition test in guinea pigs as well as the 'croton-oil-dermatitis-inhibition test' in rabbits do not permit a clear differentiation of the compounds. Significant differences in favour of CGP 14'458 - the 6α-F analogue of clobetasol propionate (CGP 9'555) - are, however, observed in the two most relevant experiments, i.e. in the HPLH and in the HVK test. The former allows to measure the degree of inhibition of mitosis and of the psoriasis-like hyperplasia produced by the action of hexadecane in the epithelium of the guinea pig skin. The HVK activity is represented in this graph as a percentage of the factor 2000 x HC. The second parameter is a measure for the intensity of the blanching reaction.

On the basis of these data and of the results of skin tolerability tests, CGP 14'458 (Ultravate®; halobetasol propionate [17]/Ultravate® [18]) has been selected for clinical testing.

Chemistry

Four different synthetic approaches to CGP 14'458 (11) were evaluated and led finally to a technically acceptable procedure. One of them [19] is presented in the Scheme.

Beclomethasone [20] (1) appeared to be an ideal starting material for the planned synthesis. The corresponding orthoester 2 generated 9β,11-epoxide 3, which was selectively hydrogenated in position 1(2) under the known conditions of homogeneous catalysis in dioxane solution, to yield the mono-ununsaturated ketone 4. Under acidic conditions the orthoester grouping was selectively hydrolyzed to the 21-hydroxy-17α-propionyloxy derivative 5. Via the 21-mesyate 6, the desired 21-CI compound 7 was formed. The introduction of an F-atom into position 6α was achieved by fluorination of the enol ether 8. Under the influence of FClO₃ in THF/H₂O besides a small amount of the 4-F isomer, unexpectedly, the 6α-F compound 9 was formed directly [20]. The opening of the epoxide proceeded smoothly under the treatment of 9 with a mixture of HF and urea, forming thus the fluoro hydrine 10 (= CGP 14'457).

Finally, dehydrogenation of 10 by DDQ in dioxane generated our target compound CGP 14'458 (11). The structures of all the intermediates and of the final substance were confirmed by analysis and by spectroscopic data (cf. Exper. Part).

Contemporarily with the development of a synthetic procedure, the optimization of the vehicles for adequate cream and ointment for CGP 14'458 was pursued by our galenics department. Using a mechanical arrangement [22] as a crude model for the in vivo liberation of the active compound from the formulation, a tailor-made ointment for CGP 14'458 on a hydrocarbon base was obtained, containing more than 5% of propylene glycol.

During the last eight years the two formulations (ointment and cream) of CGP 14'458 have been clinically tested. Special attention was given to applications in cases of severe chronic psoriasis.

In one of the studies [23], in a double-blind, multicentre, dose-finding trial by dermatologists in Germany and Switzerland in 336 patients with chronic, plaque psoriasis, 126 of whom had severe symptoms, the success rates (described as 'healed' or 'marked improvement') were 77% with 0.02% CGP 14'458 ointment, 90% with 0.05% CGP 14'458 and 80% with CGP 9'555 ointment (Table). These results clearly show that the optimum therapeutic effect of a topical corticosteroid depends not only on the potency of the compound but also on the concentration of the active ingredient. In another double-blind, parallel-group trial [24], 0.05% CGP 14'458 ointment was also more effective than 0.05% CGP 9'555 ointment in 134 patients with severe, chronic, plaque psoriasis. The success rates were 96% with CGP 14'458.
ointment and 91% with CGP 9'555 ointment. Adverse effects were reported in a smaller percentage of patients treated with 0.05% CGP 14'458 ointment than in those treated with 0.05% CGP 9'555 ointment (7.5% vs. 12%).

Thus, 0.05% CGP 14'458 (Mira-corten®) ointment proved more effective than 0.05% CGP 9'555 (Dermovate®), the most potent topical corticosteroid used worldwide in clinical practice since 1974. The registration of CGP 14'458 is meanwhile pursued in various countries. It has been already introduced into the market in the United States in January 1991 by Westwood-Squibb Pharmaceuticals (Bristol-Myers Squibb) under the proprietary name of Ultravate® [25].

**Experimental Part**

**Chemistry** [26]

9α-Chloro-11β-hydroxy-16β-methyl-3,20-dioxopregna-1,4-diene-17α,21-diy Ethyl Orto-propionate (2). TsOH · H₂O (250 mg, 1.3 mmol) was added to a stirred suspension of beclomethasone [20] (5.00 g, 12.2 mmol) in a mixture of THF (50 ml) and ethyl ortho-formate (6.25 ml,
Table. Double-Blind Multicentre Clinical Trial [23]. 336 patients with severe chronic psoriasis.

No clinically detectable systemic adverse effects observed.

31.5 mmol). The soln. was stirred at ambient temp. for 75 min. Then, pyridine (2.5 ml, 31 mmol) and AcOEt (500 ml) were added, and the soln. was washed thrice with saline, the aq. phases were extracted with AcOEt, the combined org. phases dried (Na$_2$SO$_4$) and evaporated. 300 mg of the above crude product were twice recrystallized (CH$_2$Cl$_2$/EtOH): 200 mg of essentially pure 2, which was used in the next step without further purification. 300 mg of the above crude product were twice recrystallized (CH$_2$Cl$_2$/EtOH): 200 mg of essentially pure 2, which was used in the next step without further purification.
was chromatographed (150 g alumina II/N, CHCl₃). The first homogeneous fractions (4.7 g) yielded, after crystallization, 3.53 g of pure 3. M.p. 166-168° (αD = 35.5 (c = 0.590, CHCl₃), UV (EtOH): 250 (ε = 5750). IR (CHCl₃): 1755, 1660, 1630, 1610, 1035. 1HN-MR (CDCl₃): 0.89 (3 H), 3.93 (J = 7.4, 3 H), 1.16-1.17 (17 M), J = 7, J = 9.7, 7 H), 1.45 (s, 3 H), 1.82 (q, J = 7.4, 2 H), 3.22 (m, 1 H), 3.45 (m, 2 H), 3.86 (AB, JAB = 16.5, 2 H), 6.17 (m, 1 H); 6.22 (A of AB, JAB = 10.2, 1 H). Anal. calc. for C₃₂H₄₀O₁₀ (546.58): C 71.03, H 7.93; found: C 70.96, H 7.93.

9.B.11-Epoxy-16β-methyl-3,20-dioxopregn-4-ene-17α,21-diyl Ethyl Orthopropionate (4). A solution of 3 (5.20 g, 7.01 mmol) in dioxane (250 ml) was hydrogenated in the presence of RhCl₃(PPh₃)₃ (0.52 g, 0.1 mmol), until no more H₂ was absorbed (15 h). The soln. was evaporated in vacuo, the brownish crystalline residue dissolved in CH₂Cl₂ and filtered through alumina II/N (70 g). Crystallization of the crude fractions (3.5 g) from CH₂Cl₂/Et₂O/hexane yielded pure 4 (2.26 g; additional amount of the substance was recovered from the evaporation of the crystallization solvent). M.p. 181-184°, [α]D = -10.0 (c = 0.810, CHCl₃), UV (EtOH): 242 (ε = 14440). IR (CHCl₃): 1725, 1655, 1620, 1032. 1HN-MR (CDCl₃): 0.88 (3 H, 0.96 δ (J = 7, 3 H), 1.15 (m, 7 H), 1.43 (s, 3 H), 3.44 (m, 1 H), 3.48 (m, 2 H), 3.89, 4.02 (AB, JAB = 16.65, 2 H), 5.8 (s, 1 H). Anal. calc. for C₃₂H₄₀O₁₀ (546.58): C 70.71, H 7.85; found: 70.41, 7.81.

9.B.11-Epoxy-21-hydroxy-16β-methyl-3,20-dioxopregn-4-ene-17α,21-diyl Ethyl Orthopropionate (5). A suspension of (4.30 g, 6.55 mmol) in EOH (150 ml) and 8.8 ml of a soln. of oxalic acid (2.0 g, 6.9 mmol) in H₂O (20 ml) were stirred at 40° for 40 min. The mixture was poured on ice-cold sat. NaHCO₃ (85 ml) and stirred for additional 5 min. Washing (CHCl₃) yielded crude 5 which was used without purification in the next step. An anal. sample was recrystallized from CH₂Cl₂/EtOH (1:1). M.p. 181-184°. [α]D = 0.874, c = 0.465, CHCl₃). UV (EtOH): 237 (ε = 16720), IR (CHCl₃): 3600, 1738, 1675, 1640, 1190, 1070. 1HN-MR (CDCl₃): 0.95 (3 H, c = 3, 18 H), 1.18 (J = 7.9, 3 H), 1.39 (d, J = 6.9, 3 H), 1.44 (s, 3 H), 1.81 (m, 3 H), 3.49 (m, 2 H), 3.89, 4.02 (AB, JAB = 16.65, 2 H), 5.8 (s, 1 H). Anal. calc. for C₃₂H₄₀O₁₀ (546.58): C 70.71, H 7.85; found: 70.41, 7.81.

9.B.11-Epoxy-21-mesyloxy-16β-methyl-3,20-dioxopregn-4-ene-17α,21-diyl Propionate (6). To a soln. of crude 5 (170 mg, 0.40 mmol) in pyridine (1.7 ml) and CH₂Cl₂ (0.85 ml) was added at 4° McAl (0.054 g, 0.70 mmol). The mixture was kept for 16 h at 4°, then diluted with CHCl₃ and subsequently washed with ice-cold 2N HC₁₂, sat. NaHCO₃ and H₂O. The ammonium product was crystallized, (CHCl₃/acetone/Et₂O) to give a yellowish product (2.00 g, 0.97 M). (αD = +4.15 (c = 0.431, CHCl₃), UV (EtOH): 143 (ε = 13420), IR (CHCl₃): 1735 (ester + ketone), 1665, 1620, 1535, 1156, 1040. 1HN-MR (CDCl₃): 0.90 (3 H, δ = 3, 18 H), 1.19 (J = 7.4, 3 H), 1.36 (d, J = 6.9, 3 H), 1.44 (s, 3 H), 2.30 (2 AB, JAB = 16.52, 10.5 Hz), 5.18 (s, 1 H); MS (CH₂Cl₂): 404 (34%), 434 (19 [%]HOCOEt°), 419 (43 [%]HOCOEt°), 315 ([MCOC₂H₄CH₂OH]*, 297 (1315-H₂O*)), etc. Anal. calc. for C₃₂H₃₉O₁₂S (708.63): C 61.40, H 7.13, S 0.60; found: C 61.72, H 7.21, S 6.28.

21-Chloro-9β,11-epoxy-16β-methyl-3,20-dioxopregn-4-ene-17α,21-diyl Propionate (7). LiCl (41.7 g) and anh. DMF (280 ml) were added to a soln. of 6 (16.93 g) in acetone (200 ml) and heated in an autoclave for 3 h to 80°. The cooled mixture was poured on ice, 21-chloro-9β,11-epoxy-16β-methyl-3,20-dioxopregn-4-ene-17α,21-diyl Propionate (7), LiCl (41.7 g) and anh. DMF (280 ml) were added to a soln. of 6 (16.93 g) in acetone (200 ml) and heated in an autoclave for 3 h to 80°. The cooled mixture was poured on ice, 21-chloro-9β,11-epoxy-16β-methyl-3,20-dioxopregn-4-ene-17α,21-diyl Propionate (7), LiCl (41.7 g) and anh. DMF (280 ml) were added to a soln. of 6 (16.93 g) in acetone (200 ml) and heated in an autoclave for 3 h to 80°. The cooled mixture was poured on ice, 21-chloro-9β,11-epoxy-16β-methyl-3,20-dioxopregn-4-ene-17α,21-diyl Propionate (7), LiCl (41.7 g) and anh. DMF (280 ml) were added to a soln. of 6 (16.93 g) in acetone (200 ml) and heated in an autoclave for 3 h to 80°. The cooled mixture was poured on ice, 21-chloro-9β,11-epoxy-16β-methyl-3,20-dioxopregn-4-ene-17α,21-diyl Propionate (7). 21-Chloro-9β,11-epoxy-16β-methyl-3,20-dioxopregn-4-ene-17α,21-diyl Propionate (7). 21-Chloro-9β,11-epoxy-16β-methyl-3,20-dioxopregn-4-ene-17α,21-diyl Propionate (7). 21-Chloro-9β,11-epoxy-16β-methyl-3,20-dioxopregn-4-ene-17α,21-diyl Propionate (7).

Pharmacology [27] Dermatopharmacological Tests Used for Evaluation:

UVDH (= UV-induced dermatitis inhibition assay in guinea pigs) [28]: Experimental model of inflammation produced by unfiltered UV irradiation of the depilated flanks of guinea pigs. The overall degree of inflammation inhibition elicited by 3 concentrations of a substance at various inspection times allows to rank the tested compounds in order of their acute antiinflammatory effect.

DQZMR = mean of all the individual values (dose/time) of the intensity of the inhibitory effect in % of maximum possible score.

CDH (= Croton oil - induced ear edema inhibitory assay in rabbits) [29]: Experimental model of dermatitis induced in the ear of the rabbit by the application of a soln. of croton oil. It reveals clearly differentiable inhibitory effects on the rise in skin temperature, the oedema and the increase in the tissue mass due to the inflammatory process.

APQ = antiphlogoscutal effect,
MPG = mean tissue phlogostatic activity.

HPLH (= Epidermal hyperplasia inhibition assay in guinea pigs) [30]: Allows the evaluation of the inhibitory effect of test compounds on hexadecane-induced hyperplasia of the guinea pig skin. This mimics the therapeutically useful suppressant effect on exaggerated proliferative processes in the epidermis.

DER = reduction of the superficial epithelium tissue,
MIR = reduction of the mitosis in the basal layer of the stratum corneum.

HVK (= Human skin vasoconstriction assay) [8][31]: Cutaneous vasoconstriction assay performed on healthy volunteers by application of 5 serial dilutions (10^-3 to 10^-7 w/w) of the test compounds dissolved in EtOH under occlusion to the volunteers' forearms under double-blind conditions. Visual skoring of the skin blanching is statistically evaluated after removal of the oedema.

GUSTU = average of the mean active dose - hydrocortisone (HC) - index value in % of the inhibitory effect of 2000 X HC.
GDRG = overall average of the reactivity degrees in % of the theoretical maximum (measure for the intensity of the activity).

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[1] Presented in part at the Meeting of the Swiss Chemical Society in Bern, on October 19, 1984.

[14] Coefficients \( A_F \) for the most important substituents were, \( 6a\)-F: 0.70; \( 9\alpha\)-F: 0.00; \( 9\alpha\)-Cl: 0.00; 16\beta-CH\(_3\): +0.72; 17\alpha-OCOC\(_6\)H\(_5\) +0.55; 17\alpha-OCOC\(_6\)H\(_5\) (m): +0.72; 17\alpha-OCOC\(_6\)H\(_5\) (p): 0.00; 20-OCH\(_3\) (ester): -1.05.
[16] The international nonproprietary name.
[18] Introduced on the U.S. market by Westwood-Squibb Pharmaceuticals (Bristol-Meyers Squibb Co.).
[19] Additional procedures will be described in the full paper.
[21] In analogous enol ethers or enol acetates carrying a hydrogen or an H-atom in \( 9\alpha\)-position, the fluorination occurs preferentially from the \( \beta \)-side. To obtain the desired \( 6a\)-F derivative, the compound has to be equilibrated under relatively vigorous conditions.