EXPERIENTIA

Vol. 34 - Fasc. 9 Pag. 1105-1246 15.9.1978

GENERALIA

Retinoids, a new class of compounds with prophylactic and therapeutic activities in oncology and dermatology*

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Summary. A review of recent investigations in the retinoid field is presented. Retinoic acid exerts a prophylactic and a therapeutic effect on chemically induced benign and malignant epithelial tumors in mice. In clinical studies positive therapeutic results have been obtained in patients with preneoplastic and neoplastic epithelial lesions. However, treatment with retinoic acid is limited by serious side effects (hypervitaminosis A syndrome). Therefore, the synthesis of analogs of retinoic acid (retinoids) possessing a more favorable therapeutic ratio has been initiated. Among a large series of synthesized compounds, certain aromatic analogs proved to have a particularly favorable therapeutic ratio. The structure-activity relationship of the most active retinoids is discussed including some biological data concerning prophylaxis and therapy of epithelial tumors. The total synthesis of retinoids according to various building schemes is discussed in detail. Methods for the synthesis of the cyclic end group, of the polyene chain component, and of the full retinoid skeleton are described. Metabolic studies of retinoic acid and of the most active retinoid, as well as the synthesis of some isolated metabolites are outlined. Suggestions concerning the mechanism of action of retinoids are made. Some clinical results on the treatment of acne, psoriasis and precancerous conditions are reported.

Introduction

In 1909 Stepp[2,3] first described a fat-soluble principle which proved essential for life. Stepp carried out experiments on extracted 'lipid-free' diets and was able to show that the extracted food did not allow mice to survive unless an extract from egg yolk was added. Shortly afterwards, other nutritionists[4-8] were able to confirm these results. They discovered additional animal sources of the active principle, i.e. butter-fat and cod liver oil. In 1920 Drummond[9] suggested that the novel fat-soluble 'accessory factor'[4], also first designated as 'fat-soluble A'[10], should be called vitamin A.

These fundamental findings formed the starting point of vitamin A research which, in the following years, has developed into a field of great importance.

Some highlights may be mentioned: In 1931 Karrer et al.[11,12] were able to obtain vitamin A preparations of high purity and activity from fish liver oils and to propose the correct structural formula for vitamin A. In 1942 Baxter and Robeson[13,14] succeeded in crystallizing pure vitamin A and several of its esters. The first total synthesis of crystalline vitamin A was announced by Isler et al.[15,16] in 1947, and soon afterwards an industrial manufacturing procedure was developed. Many other outstanding scientists have contributed to the chemical, biological and clinical investigation of the vitamin during the past decades. The tremendous amount of scientific facts collected over the years has been the subject of numerous monographs and review articles[17].

Today, vitamin A is well known to everybody for its importance in general growth, the growth and differentiation of epithelial tissues, visual function and reproduction[26,30-33]. In general, the term 'vitamin A' is now used when reference is made to the biological activity of more than one vitamin A-active substance[33]. Figure 1 shows the structural formulas of 3 of the

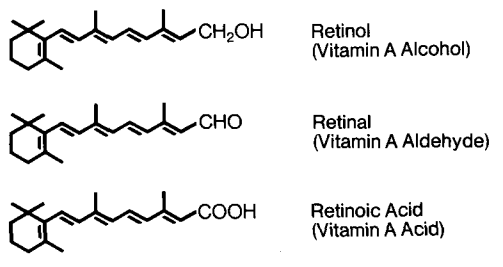


Fig. 1. Vitamin A compounds.

* Based on a lecture presented at the Symposium on Horizons in Medicinal Chemistry, Centennial ACS Meeting, New York, 6 April, 1976[1].

more important vitamin A compounds: retinol (vitamin A alcohol), retinal (vitamin A aldehyde) and retinoic acid (vitamin A acid). All 3 compounds contain as common structural units a trimethylcyclohexenyl group and an all-trans-configurated polyene chain with 4 double bonds. They are beautifully crystalline substances of limited stability.

Vitamin A compounds and cancer

The relationship between vitamin A compounds and cancer - although not as generally known as the properties mentioned - has also attracted the interest of scientists as early as 1926, when Fujimaki[34] detected the development of carcinomas in the stomach of rats which were fed a vitamin A-deficient diet. In animal experiments it had furthermore been noticed that vitamin A-deficiency leads to hyperkeratosis of the skin and to metaplastic changes in the epithelia of the gastrointestinal, the respiratory and the urogenital tract[30,35-38]. These metaplasias may be considered as the first step in the transformation process from a normal to a neoplastic tissue. In fact, in the later 1960's several authors[39-42] were able to show that the induction of benign and malignant epithelial tumors in animals could be retarded or even prevented by systemically applied retinol or retinyl palmitate, and recently Bollag[43] demonstrated that systemically applied retinyl palmitate also exerts a therapeutic influence on established skin papillomas of mice. However, retinol and its esters have one major disadvantage: they are stored in the liver, mainly as the palmitate[44], and therefore, when given in higher doses, interfere markedly with the levels of the compounds in blood, tissues and liver, causing certain toxic effects known under the name of the hypervitaminosis A syndrome[45]. Retinoic acid, however, is not stored in the liver or in other tissues, it very rapidly disappears from the animal body and also does not undergo reduction to retinol in vivo[46]. Furthermore, it has been shown by several investigators[47-49] that topically applied retinoic acid can be used more successfully than retinyl palmitate in certain dermatologic disorders, e.g. ichthyosis, psoriasis and acne. Therefore, we decided to concentrate in our experiments on retinoic acid and not on retinol or its esters.

Therapeutic experiments with retinoic acid. Skin papillomas and carcinomas

As a model, we have chosen chemically induced papillomas and carcinomas of the skin of mice. As an initiator, 7,12-dimethyl-benzanthracene was used, which was painted (2 × 150 µg) on days 1 and 15 on the back skin of female Swiss albino mice, followed by croton oil (500 µg, 2 × weekly) as promoter[43,50-52]. Papillomas mostly appeared after 3-8 months, whereas carcinomas were not induced until after 5-12 months. Figure 2 shows one of the animals thus prepared for a therapeutic papilloma experiment. The well-developed papillomas can be seen spread all over the back skin of the animal.

In a typical therapeutic papilloma experiment (table 1) carried out with this model, the control animals showed an increase of the papilloma diameter per animal of 22.7% within 14 days, whereas with the animals treated with retinoic acid a regression of up to 51.4% was observed. Furthermore, even chemically induced skin carcinomas responded to a certain degree with regressions[50,51]. On the other hand, the growth of transplantable tumors was not at all inhibited[50,51]. This is in contrast to all cytotoxic agents used in today's cancer chemotherapy which exert an influence on transplantable tumors, but none on chemically induced skin papillomas or carcinomas. Thus, the mode of action is probably very different from that of the compounds used up to now in the chemotherapy of tumors.

Clinical results with retinoic acid

After these positive animal experiments we tried to transfer the results obtained to clinical therapy. In table 2 are recorded some results which have been

Table 1. Therapy of chemically induced papillomas with retinoic acid

Dose (mg/kg)	Average sum of the papilloma diameters per animal (mm)		Change in the average sum of the papilloma diameters per animal
	Day 0	Day 14	
Controls	23.4	28.7	+22.7%
Retinoic acid (1 × weekly i.p.)			
100	26.3	21.5	−18.2%
200	33.2	21.3	−35.8%
400	28.6	13.9	−51.4%

Table 2. Therapy of actinic keratoses and basal cell carcinomas with retinoic acid

	No. of cases	Complete regression	Partial regression	No change
Actinic keratoses	60	24 (40%)	27 (45%)	9 (15%)
Basal cell carcinomas	16	5 (31%)	10 (63%)	1 (6%)

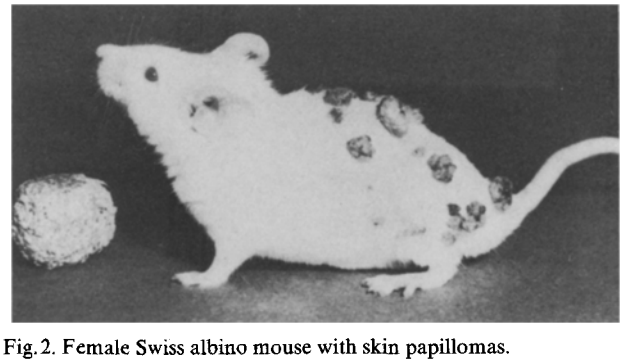


Fig. 2. Female Swiss albino mouse with skin papillomas.

obtained by the local treatment with retinoic acid of actinic keratoses, a precancerous condition, and of basal cell carcinomas[53,54]. With 40% of the patients having actinic keratoses complete regression, and with 45% partial regression was observed. With patients having basal cell carcinomas, the corresponding figures amounted to 31% and 63%, respectively.

Positive clinical results have also been obtained by the oral treatment with retinoic acid. In this case, papillomas of the urinary bladder have been favorably influenced, as seen in table 3. Out of 33 cases 10 showed complete, and 12 showed partial regression[55,56].

Hypervitaminosis A and the therapeutic ratio

Although from a scientific point of view the reported results appear encouraging, this treatment cannot be recommended for practical purposes because retinoic acid also induces, in animals as well as in man, the toxic effects of the hypervitaminosis A syndrome[45] mentioned earlier. In man, the main symptoms are alterations of the skin (e.g. erythema, desquamation, hair loss) and mucous membranes (e.g. cheilitis, stomatitis, conjunctivitis), hepatic dysfunction and headache. All these symptoms prohibit the use of higher doses which may be necessary to treat successfully precancerous conditions and particularly carcinomas. Therefore, the synthesis of retinoic acid analogs has been initiated aiming at compounds which would hopefully possess high activity and high tumor specificity together with low toxicity.

To this end, a new screening system has been developed and applied which now allows to detect a dissociation between the antitumor effect and the hypervitaminosis A syndrome. The therapeutic ratio has been defined[57,58] (table 4) as the ratio between the dose given i.p. once a week during 2 weeks, causing a 50% regression of papillomas and the lowest daily i.p. dose (14 days' study), causing a defined degree of hypervitaminosis A. In mice, the latter becomes manifest in the form of weight loss, desquamation of the skin, hair loss and bone fractures. A grading system of 0–4 - none to very marked - for each of the above-mentioned symptoms was used. The hypervitaminosis A was defined as being that condition of the animals when the addition of all the symptom grades yielded at least 3. Thus, the therapeutic ratio enables us to compare specific retinoic acid analogs with each other.

Table 3. Therapy of papillomas of the urinary bladder with retinoic acid

No. of cases	Complete regression	Partial regression	No change	Progression
33	10	12	7	4

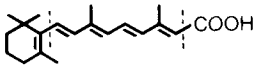
Table 4. The therapeutic ratio of retinoic acid analogs

Papilloma effect	Hypervitaminosis A symptoms	
	Weight loss	0–4
	Desquamation of the skin	0–4
	Hair loss	0–4
	Bone fractures	0–4
Weekly dose causing 50% regression of papillomas	Lowest daily dose causing hypervitaminosis A symptoms (3)	

$$\frac{\text{Papilloma: } ED_{50}/\text{week}}{\text{Hypervitaminosis A: Minimal dose/day}}$$

Retinoic acid analogs (retinoids)

As was recently suggested by Sporn[59], retinoic acid analogs are now called retinoids, analogous to the naming of carotenoids or steroids, including both the natural forms and the synthetic analogs of vitamin A. Figure 3 demonstrates that the retinoic acid molecule is composed of 3 main building units, namely of the cyclic end group[60], of the polyene chain and of the polar end group. Following the principles of medicinal chemistry, each of these components may be modified so that an almost unlimited number of retinoids would result, at least in theory. But why not take as a model for molecular modifications Nature's ingenuity for structural design? For example, Nature has produced a rich variety of colors, and, since the earliest days, man has been intrigued by the beautiful pigments present in living organisms. Of the various classes of natural pigments, the carotenoids are among the most widespread and important[61]. They are responsible for many of the brilliant yellow and red colors in flowers and fruits. Here, Nature has created many interesting structures[62,63] as illustrated by a few examples in figure 4.



Cyclic End Group Polyene Chain Polar End Group

Fig. 3. Components of the retinoic acid molecule.

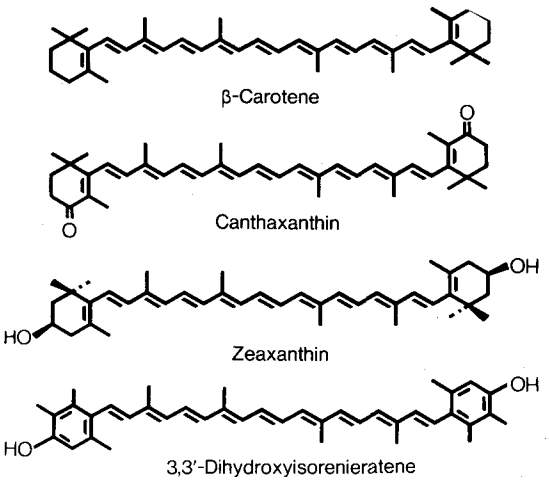


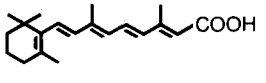
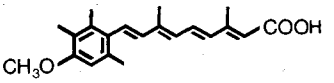
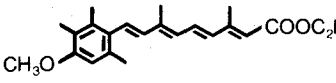
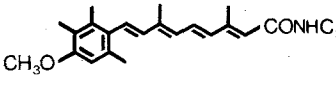
Fig. 4. Some natural carotenoids.

These compounds are not only important natural pigments – some of them are now commercially synthesized[25] and applied as pigmenters in food and feed[64] – but they also offer the organic chemist many useful cyclic end group modifications for the synthesis of retinoids. Thus, retinoic acid, a natural retinoid, contains a trimethylcyclohexenyl ring which is identical with the cyclic end group of the most important provitamin A compound β-carotene (figure 4) and its metabolite retinol, respectively. It is interesting to note that retinoic acid acts as a partial vitamin A in that it can promote normal growth but not carry out the specialized functions of maintaining vision[46] or reproduction[65]. It is obtained by an irreversible enzymatic oxidation of retinal[66] which, in turn, is formed in the intestinal wall from carotenoids (provitamins A)[67,68] or from retinol[69,70]. In other cases, Nature has produced alicyclic rings with oxygen-containing functional groups (e.g. canthaxanthin and zeaxanthin) or substituted phenyl rings (e.g. 3,3′-dihydroxyisorenieratene) (figure 4). It was this aromatic carotenoid, synthesized by us some time ago[71], which had helped us in finding one of the most active cyclic end groups so far.

From a large series of compounds a selection of some aromatic retinoids synthesized by Rüegg and Ryser[72] together with their respective experimental numbers and therapeutic ratios in comparison to retinoic acid is shown in table 5. These retinoids proved to be particularly active preparations possessing a 10 times more favorable therapeutic ratio than retinoic acid itself. These compounds carry an aromatic p-methoxy-trimethylphenyl ring and, as a polar end group, a carboxy, ethoxycarbonyl and ethyl amide group, respectively. The polyene chain is the same as in retinoic acid.

In table 6 are listed some retinoids bearing chlorine atoms in the aromatic ring which also exhibit similar therapeutic ratios. It is interesting to note that with the last 3 of these the dose causing a 50% regression of papillomas was quite low, the toxicity, however, was correspondingly high.

Table 5. Therapeutic ratios of some aromatic retinoids

Ro-No.	Chemical Structure	Therapeutic Ratio
1-5488		$\frac{400}{80} = 5$
10-1670		$\frac{50}{100} = 0.5$
10-9359		$\frac{25}{50} = 0.5$
11-1430		$\frac{50}{100} = 0.5$

The synthesis and biological activity of various dihydroretinoic acids and their derivatives have recently been reported[73]. Some examples are shown in table 7. Other molecular modifications have been less successful. Thus, compounds carrying an α-cyclohexenyl ring, an acyclic end group, a 5-membered ring or an altered side chain (missing methyl groups, thio esters, additional carbonyl group) did not reach satisfactory therapeutic ratios. Some examples are listed in table 8.

Therapeutic and prophylactic experiments with the aromatic retinoid Ro 10-9359

The aromatic retinoid Ro 10-9359 possessing a p-methoxy-trimethylphenyl ring has turned out to be a compound of considerable interest. In a therapeutic papilloma experiment (table 9) carried out with Swiss albino mice, the controls showed a progression of the chemically induced papillomas by 23.2%, whereas in the animals treated with Ro 10-9359 the papillomas

Table 6. Therapeutic ratios of some chlorinated aromatic retinoids

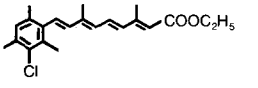
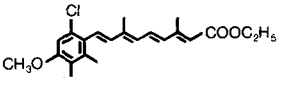
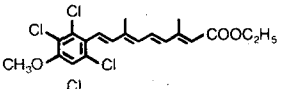
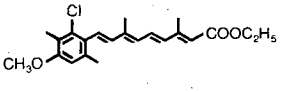
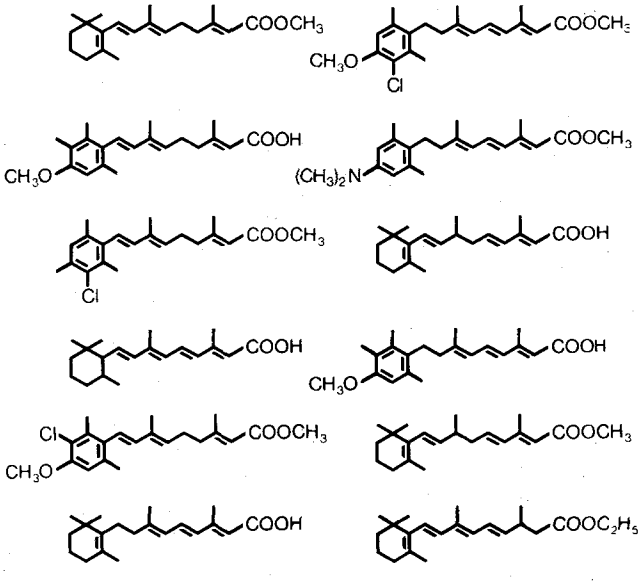
Ro-No.	Chemical Structure	Therapeutic Ratio
11-0503		$\frac{200}{400} = 0.5$
11-8579		$\frac{12.5}{25} = 0.5$
11-8284		$\frac{25}{25} = 1.0$
12-0955		$\frac{6}{12} = 0.5$

Table 7. Some dihydroretinoic acids and derivatives



regressed in a dose-dependent way. After a 2-weeks' treatment with 200 mg/kg once a week, a regression of the papilloma diameter of 74.1% was observed[57,58].
In figures 5 and 6 some chemically induced back skin papillomas of an animal can be seen before and after treatment, respectively, with Ro 10-9359.
The effect on carcinomas was also pronounced (table 10). Whereas the mean carcinoma volume of the controls increased by 92.7%, the carcinoma volume of the animals treated regressed by 53.4% and 72.2%, respectively[57,58]. The high doses used, however, were already provoking toxic effects. As an example, mouse skin carcinomas before and after treatment with Ro 10-9359 are shown in figures 7 and 8, respectively.
In a recent study it could be demonstrated that the retinoid Ro 10-9359 has also a very marked prophylactic effect on the development of papillomas and carcinomas of mouse skin when applied systemically during the promotion phase of chemical carcinogenesis[80]. Thus, in an experiment outlined in table 11 the controls showed a mean number of papillomas per

Table 8. Some other molecular modifications of retinoic acid (* = references)

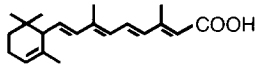
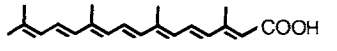
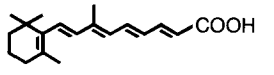
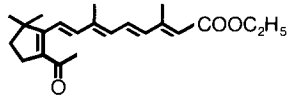
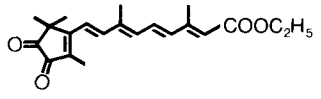
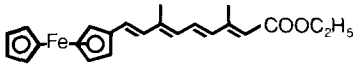
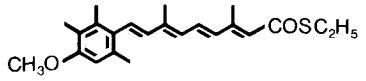
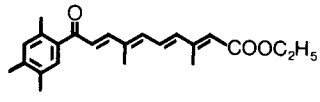
Ro-No.	Chemical Structure		Therapeutic Ratio
8-5664		[74]*	$\frac{200}{25} = 8$
10-0733		[75]	$\frac{400}{400} = 1$
8-9546		[76]	$\frac{200}{100} = 2$
8-9372		[77]	$\frac{200}{25} = 8$
11-9588		[78]	$\frac{150}{200} = 0.75$
13-0028		[79]	$\frac{100}{50} = 2$
12-7161		[78]	$\frac{50}{50} = 1$
11-9591		[76]	$\frac{100}{50} = 2$

Table 9. Therapy of chemically induced papillomas with Ro 10-9359

Dose (mg/kg)	Average sum of the papilloma diameters per animal (mm)		Change in the average sum of the papilloma diameters per animal
	Day 0	Day 14	
Controls	21.1	26.0	+23.2%
Ro 10-9359 (1 × weekly i.p.)			
12.5	24.8	17.3	−30.2%
25	24.4	12.5	−48.8%
50	20.4	9.1	−55.4%
100	28.0	8.5	−69.6%
200	25.5	6.6	−74.1%

Table 10. Therapy of chemically induced skin carcinomas with Ro 10-9359

	Mean carcinoma volume (mm^3)		Change in volume
	Day 0	Day 14	
Controls	600.1	1156.4	+92.7%
Ro 10-9359 (daily i.p.)			
400 mg/kg	525.5	146.3	−72.2%
200 mg/kg	619.3	288.9	−53.4%

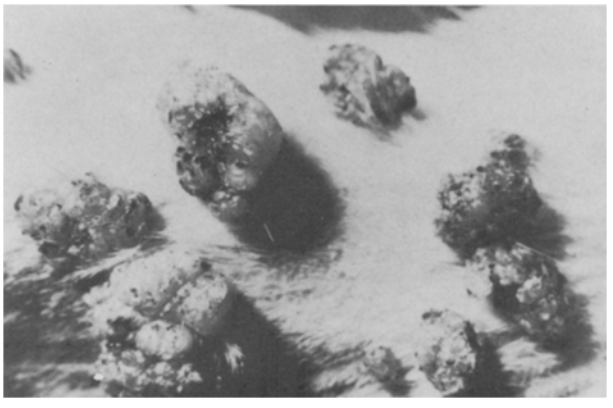


Fig. 5. Chemically induced mouse back skin papillomas before treatment with Ro 10-9359.

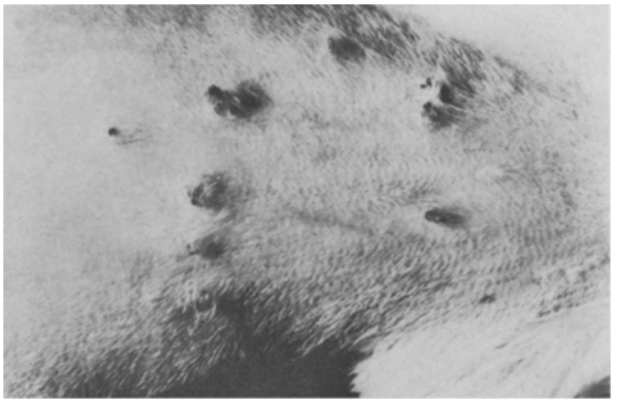


Fig. 6. Chemically induced mouse back skin papillomas after treatment with Ro 10-9359.

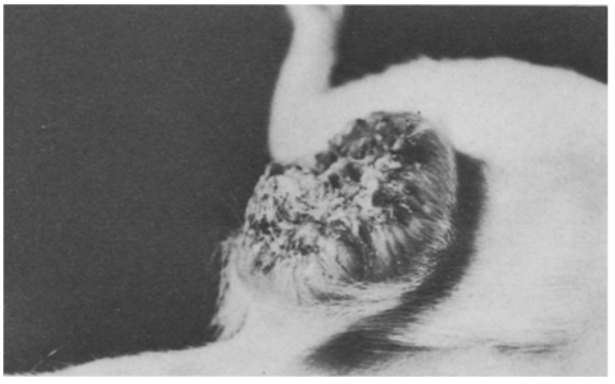


Fig. 7. Mouse skin carcinoma before treatment with Ro 10-9359.

Fig. 8. Mouse skin carcinoma after treatment with Ro 10-9359.

Table 11. Prophylaxis of chemically induced papillomas and carcinomas with Ro 10-9359

	Day	No. of mice	Papillomas Mean No. per mouse	Mean volume per mouse (mm^3)	Carcinomas Number (cumulative number)
Controls	293	47/66	12.4	426.3	46
Ro 10-9359 30 mg/kg orally daily	293	56/66	1.1	6.8	4

animal of 12.4 at day 293, whereas in the treated group this number had reached only 1.1. The mean volume of papillomas per animal showed a still more marked difference between the controls and the treated animals in that the volumes amounted to 426 mm^3 and 6.8 mm^3, respectively. In the case of the carcinomas, the treatment had a clear-cut preventive effect, as only 4 carcinomas appeared in the treated group compared to 46 in the controls.

We may conclude that the aromatic retinoid Ro 10-9359 has a marked therapeutic and prophylactic effect on chemically induced benign and malignant epithelial tumors. With respect to the papilloma- and carcinoma-regressing effect, it is superior to retinoic acid as reflected in a 10 times better therapeutic ratio.

It is interesting to note in this connection that despite this very marked activity against chemically induced skin tumors, Ro 10-9359 was similarly inactive as retinoic acid when tested against transplantable tumors (with the exception of the transplantable rat chondrosarcoma[88]). This investigation has also proved that the antitumor effect is not strictly linked with the development of hypervitaminosis A and that a dissociation between these 2 properties leads to a broader therapeutic margin.

In the last 2 years several interesting papers have been published showing also either a prophylactic or a therapeutic effect of various retinoids on metaplasias, preneoplastic and neoplastic lesions[81-95]. These investigations have been carried out in different model systems, in vitro as well as in vivo.

Synthesis of retinoids

For the synthesis of retinoids well-established methods of modern polyene chemistry[25] are employed. The general synthetic principle is outlined in figure 9.

A suitable phosphonium salt **1** or an aldehyde **2** is required which is combined with a corresponding side chain component, e.g. with the C_5-aldehyde ester **3** and with the C_{10}-phosphonium salt **4**, respectively. The group R can thereby be acyclic, alicyclic, aromatic or heterocyclic. As a coupling method, the Wittig reaction is preferred, as shown by the listed examples, in others it could be a Grignard, a Reformatzkii or an acetylide addition reaction. The retinoid skeleton is thereby formed without major difficulties. In this section, syntheses of the cyclic end group, of the side chain component and of the full retinoid skeleton are summarized. To proceed in a chronological manner, the syntheses of vitamin A and retinoic acid are discussed first.

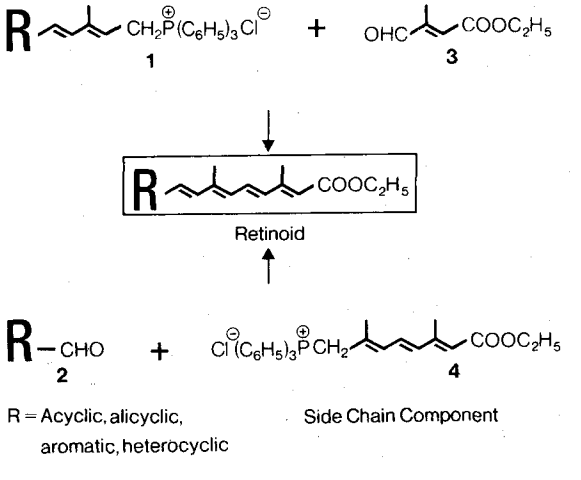
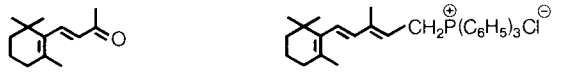


Fig. 9. General principle for the synthesis of retinoids.

One of the most important cyclic components is β-ionone (figure 10), nowadays a cheap technical intermediate, which is produced in large quantities. This compound is the starting material for all technical syntheses of vitamin A today[19,23,25]. In the Roche synthesis, developed by Isler et al.[15,16], β-ionone is transformed in 5 steps into all-trans-vitamin A acetate (retinyl acetate) in high yield and excellent quality.

Retinoic acid (figure 1) can be obtained in various ways. It was first totally synthesized by van Dorp and Arens[96,97] and was later prepared by several authors in the course of their investigations into the synthesis of retinol[25]. Utilizing vitamin A acetate (retinyl acetate) as a readily available starting material, retinoic acid can be made by a 3-step process. Thus, alkaline hydrolysis of the acetate grouping, followed by manganese dioxide oxidation of the intermediate retinol, yields the corresponding aldehyde, retinal[98] (figure 1), which is then further oxidized to the desired carboxylic acid[99,100]. This is the standard method. It has been found, however, that this sequence of reactions can be significantly facilitated and improved by oxidizing retinyl acetate directly. Surprisingly, the reagent of choice employed here proved to be silver oxide in alkaline medium affording all-trans-retinoic acid in excellent yield[101].

Another important cyclic component is represented by the C_{15}-phosphonium chloride **5** (figure 10) which is also readily available from β-ionone[102-105]. This Wittig salt is a key intermediate in the BASF syntheses of vitamin A[102-105] and retinoic acid[106].



β-Ionone 5

Fig. 10. Some alicyclic components.

The necessary C_5-, C_7- and C_{10}-side chain components are synthesized from readily available starting materials and intermediates, respectively. A selection of some well-established reaction sequences is presented in the following section.

Thus, the important C_5-building units **3**[107-109], **6**[105], **7**[105] and **8**[107-109] (figure 11) can be produced on a technical scale in a few steps from acetone.

A convenient synthesis of the C_7-aldehyde acetate **9** (figures 11 and 12) according to the scheme $C_5 + C_2 = C_7$ was first reported by Makin[110], employing an enol ether condensation reaction. This efficient chain-lengthening procedure was developed by Roche chemists[111] years ago and ever since has quite frequently been used in polyene synthesis[25]. Thus, on a technical scale, the C_5-acetal **12** (figure 12) is reacted with ethyl vinyl ether in the presence of zinc chloride yielding the intermediate acetal **13**, which is then transformed into the desired C_7-unit **9** on treatment with sodium acetate/acetic acid[112].

The synthesis of the C_{10}-aldehyde ethyl ester **11** and the corresponding methyl ester, respectively (figure 13), can be readily achieved according to the scheme $C_5 + C_5 = C_{10}$, as developed by us more than a decade ago[25]. Thus, the C_5-acetal acetate **6** is first hydrolyzed, and the primary alcohol **14** obtained is then oxidized, yielding the C_5-aldehyde **15**[105,113]. This is reacted in a Horner reaction with the C_5-phosphonate **16**, followed by hydrolysis of the resulting C_{10}-acetal ester **17**.

Another efficient procedure for the preparation of the C_{10}-aldehyde ester **11** starts from the C_5-acetal ester **8** (figure 11) which is first transformed into the C_7-component **10** by means of an enol ether condensation. The latter compound, in the form of its acetal, is then again subjected to an enol ether condensation using ethyl propenyl ether[76,102].

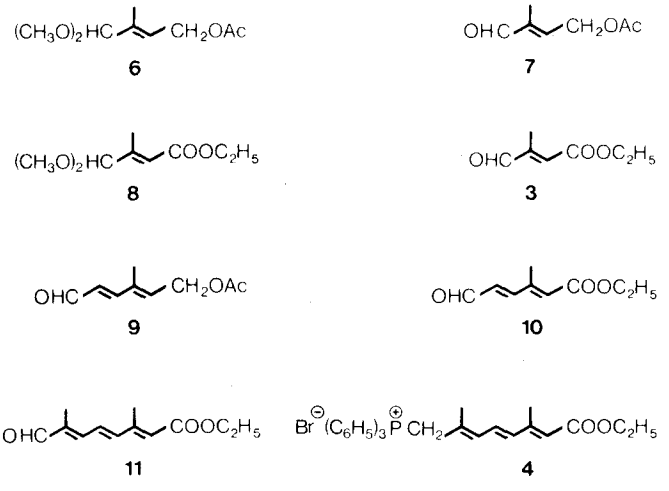


Fig. 11. Some C_5-, C_7- and C_{10}-side chain components.

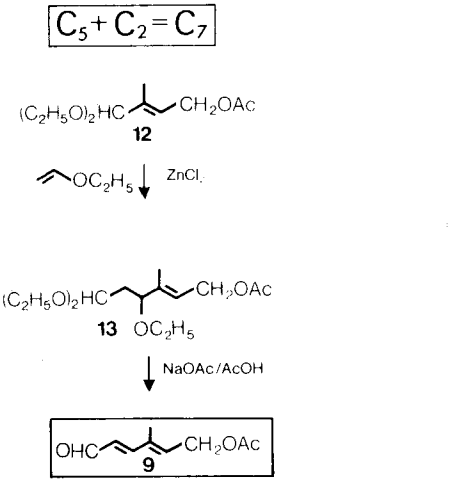


Fig. 12. Synthesis of 5-formyl-3-methyl-2,4-pentadienyl acetate (C_7-aldehyde acetate).

The C_{10}-aldehyde ester **11** represents one important C_{10}-component, the C_{10}-phosphonium salt **4** (figure 13) the other. This compound and the corresponding methyl ester have been synthesized in a simple way, namely by metal hydride reduction of the aldehyde group in **11** yielding the primary hydroxy ester **18**, which is then brominated, followed by treatment of the resulting bromide with triphenylphosphine[25,114].

Concerning the synthesis of aromatic retinoids, 2 of the more important compounds (i.e. Ro 10-9359 and Ro 11-1430) have been chosen as representative examples.

For the synthesis of the aromatic C_{10}-, C_{13}- and C_{15}-components 2,3,5-trimethylphenol is used as a readily available starting material. This compound is first transformed into the aldehyde **19**[115] (figure 14) which on reduction with sodium borohydride and subsequent treatment with $(C_6H_5)_3P/HCl$ is readily transformed into the C_{10}-phosphonium chloride **20**[79].

For the synthesis of the C_{13}-phosphonium chloride **22** (figure 14) the intermediate aldehyde **19** is condensed with acetone yielding the C_{13}-ketone **21**[115]. Subsequent metal hydride reduction leads to the corresponding secondary alcohol, which can be readily converted into the desired Wittig salt by treatment with triphenylphosphine and hydrochloric acid[112].

Reaction of the intermediate C_{13}-ketone **21** (figure 14) with vinyl magnesium chloride yields the vinyl carbinol **23** which, on treatment with triphenylphosphine

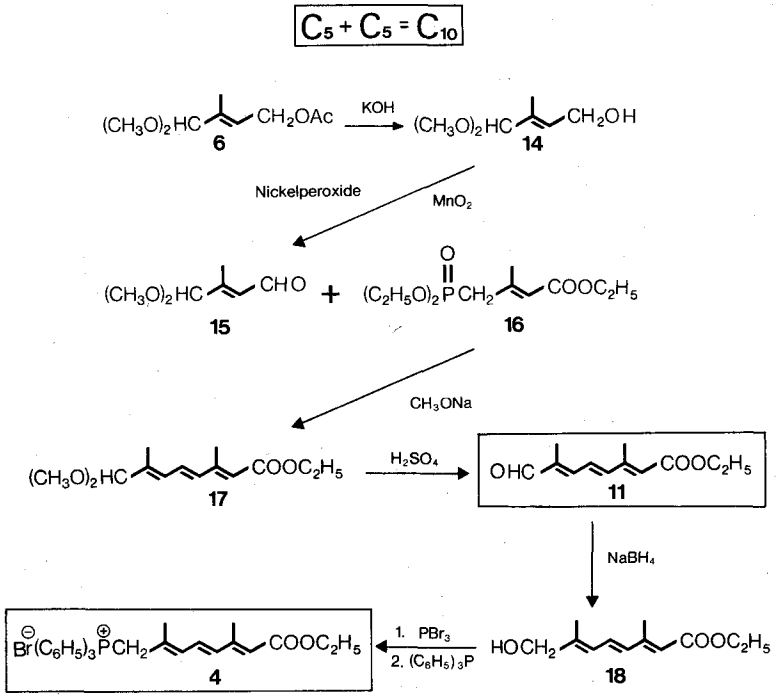


Fig. 13. Synthesis of ethyl 7-formyl-3-methyl-2,4,6-octatrienoate (C_{10}-aldehyde ester) and of 7-ethoxycarbonyl-2,6-dimethyl-2,4,6-hepta-trienyl triphenylphosphonium bromide (C_{10}-phosphonium salt ester).

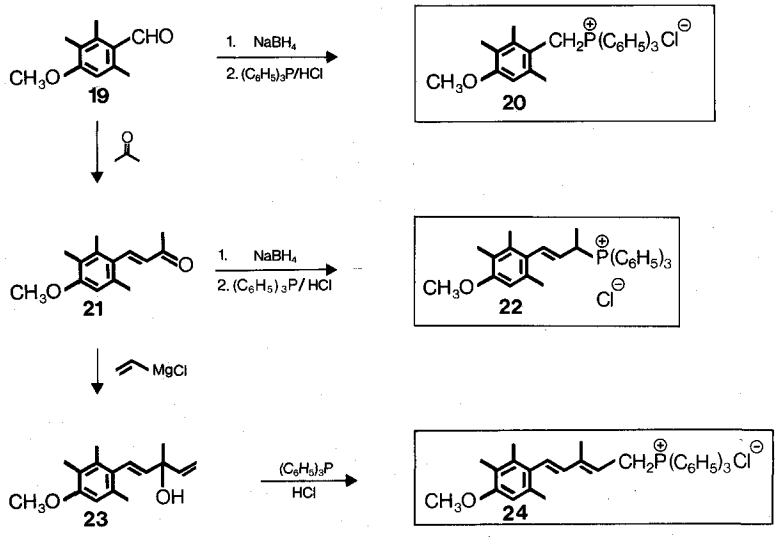


Fig. 14. Synthesis of some aromatic C_{10}-, C_{13}- and C_{15}-components.

and hydrochloric acid, is transformed into the C_{15}-Wittig salt **24**[116].

The synthesis of Ro 10-9359 and Ro 11-1430, respectively, is now achieved according to three different building schemes. In the 1st of these (figure 15) the C_{10}-phosphonium chloride **20** is reacted with the C_{10}-aldehyde ester **11** in the presence of butylene oxide, yielding the aromatic retinoid Ro 10-9359 directly[79]. In the 2nd scheme (figure 16), the C_{15}-Wittig salt **24** is condensed with the C_5-aldehyde ester **3** yielding, after isomerization, the desired aromatic ester. Subsequent saponification leads to the corresponding acid **25**, which is transformed into the corresponding ethyl amide Ro 11-1430 by standard methods. According to the scheme $C_{13}+C_7=C_{20}$ (figure 17), the C_{13}-phosphonium chloride **22** is condensed with the suitable C_7-aldehyde ester **10** to give an all-trans-/9-cis-mixture of isomeric C_{20}-esters **26** which, on saponification and isomerization, is transformed into the all-trans-acid **25**[76]. This acid can then be esterified following the known methods.

Alternatively, the C_{13}-Wittig salt **22** can be reacted with the C_7-aldehyde acetate **9**, yielding an all-trans-/9-cis-mixture of C_{20}-acetates **27** which, on alkaline oxidation and subsequent isomerization, furnishes the same acid **25**[112].

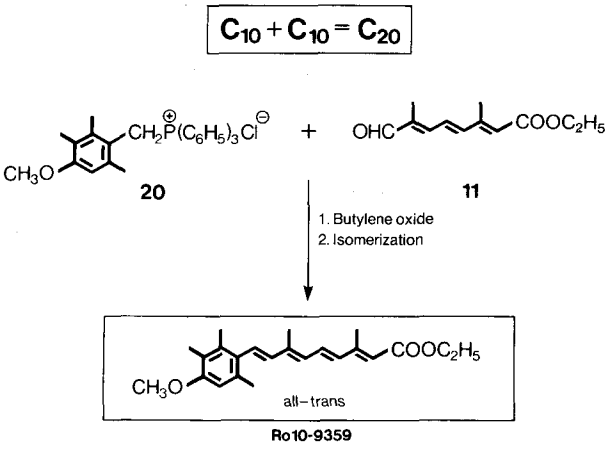


Fig. 15. Synthesis of ethyl 9-(4-methoxy-2,3,6-trimethylphenyl)-3,7-dimethyl-2,4,6,8-nonatetraenoate (Ro 10-9359) according to the scheme $C_{10}+C_{10}=C_{20}$.

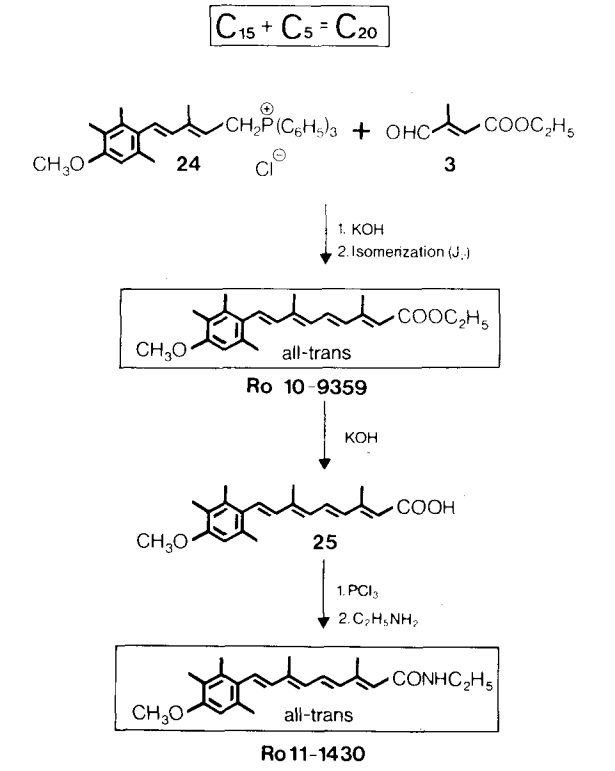


Fig. 16. Synthesis of Ro 10-9359 and of 9-(4-methoxy-2,3,6-trimethylphenyl)-3,7-dimethyl-2,4,6,8-nonatetraenoic acid ethylamide (Ro 11-1430) according to the scheme $C_{15}+C_5=C_{20}$.

Metabolism of retinoic acid

The search for metabolites of retinol and retinoic acid was intensified after there were suggestions that these compounds could be precursors of an unknown, active metabolite.

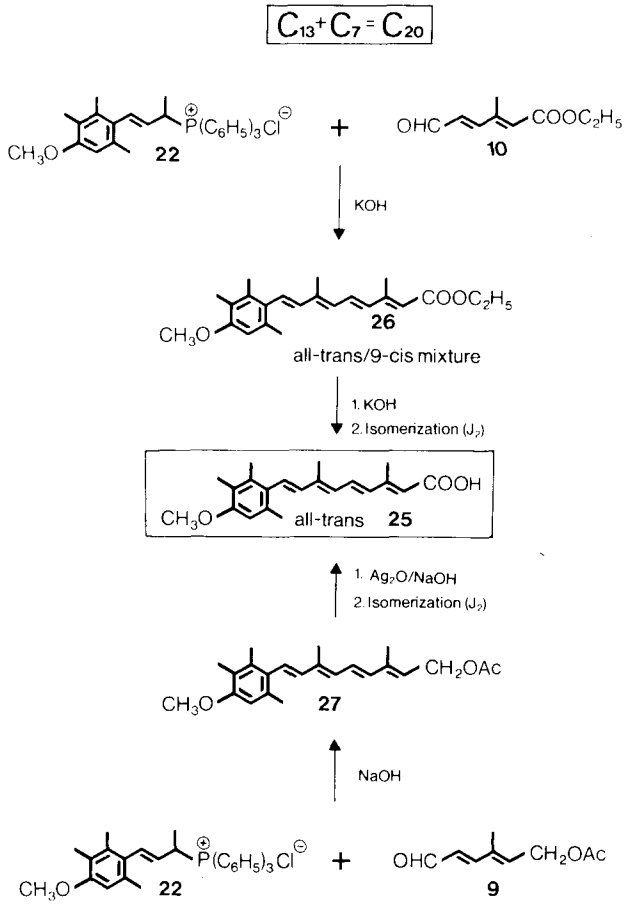


Fig. 17. Synthesis of Ro 10-9359 according to the scheme $C_{13}+C_7=C_{20}$.

After oral administration to rats, retinoic acid was rapidly excreted in the bile as retinoyl β-glucuronide (**28**)[117] (figure 18). The metabolite isolated from the livers of rats fed large doses of retinoic acid has been identified as 13-cis-retinoic acid (**29**)[118]. Numerous publications concerning retinoic acid metabolites in rat urine have appeared in the literature but none of these metabolites were well characterized or identified. As a result of experiments done with radioactively-labelled retinoic acid, it was suggested that the metabolites in the urine of rats had a shortened side chain[119]. In 1974 4 urinary metabolites were isolated after injection of retinoic acid to rats and humans[120]. Structures **30**–**33** (figure 18) have been suggested for these compounds which contain an oxidized cyclohexene ring and a partially hydrogenated side chain. Recently, three major urinary metabolites (**34**, **35** and **36**, figure 18) have been isolated and their structures elucidated after i.p. administration of high doses of 15-[^{14}C]- and 10,11-[^{3}H]-retinoic acid (figure 19) to rats[121]. It was shown that these metabolites also contain a cyclohexenone moiety, but the side chain was shortened by 4 C-atoms yielding a C_{16}-skeleton. From the faeces, 3 major metabolites (**32**, **37** and **38**, figure 20) and the intact compound were isolated, characterized and identified following i.p. administration of high doses of 15-[^{14}C]- and 10,11-[^{3}H]-retinoic acid to rats[122]. The reactions leading to these faecal metabolites comprised oxidation of the cyclohexene ring in position 4, hydroxylation of the 5-methyl group, and 9-trans/cis isomerization of the tetraene side chain.

Metabolism of the aromatic retinoid Ro 10-9359

The metabolism of the aromatic retinoid Ro 10-9359 has been investigated in the rat and in man[123]. Following oral administration of the tritium-labelled drug 10,11-[^{3}H]-Ro 10-9359 (figure 19) to rats, the unchanged drug could be found in the plasma and in the

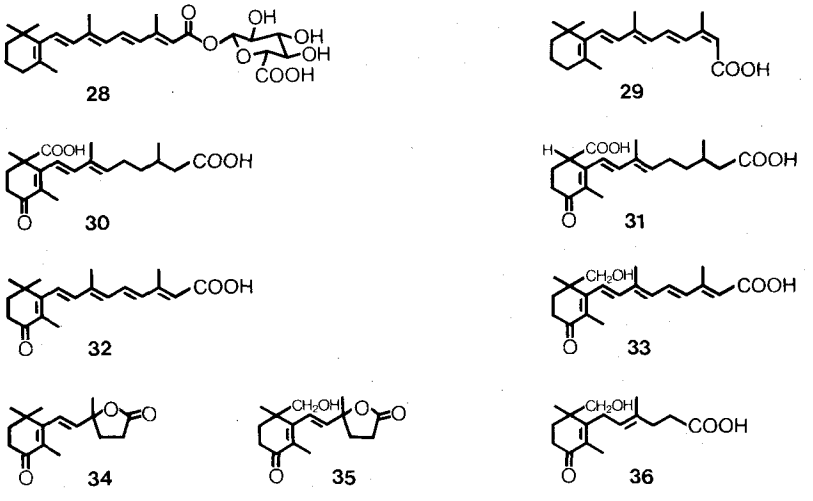


Fig. 18. Urinary metabolites of retinoic acid.

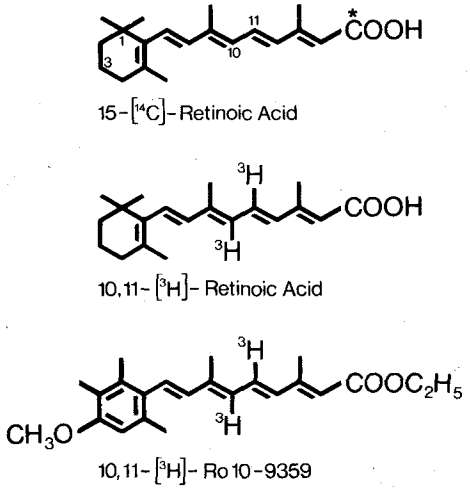


Fig. 19. Labelled compounds used for metabolic investigations.

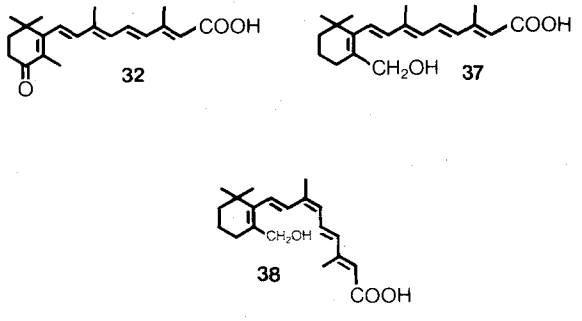


Fig. 20. Faecal metabolites of retinoic acid.

faeces. The corresponding methoxy acid **39** (figure 21) could be detected in the plasma and also in the bile as a conjugate (**40**). Also from the bile, the corresponding phenolic acid **41** and the hydroxymethyl compound **42** were isolated as a conjugate. In the urine, 2 main metabolites were found, namely the phenolic C_{16}-acid **43** and the corresponding γ-lactone **46**.

The metabolism of Ro 10-9359 in man was studied with patients suffering from generalized psoriasis who have been treated with this compound. After oral administration of therapeutic doses of 10,11-[^{3}H]-Ro 10-9359 to psoriasis patients, the unchanged drug and the corresponding methoxy acid **39** could be found in the plasma (figure 21). In addition, the unchanged drug was detected in the faeces of the patients. From the urine, a series of acidic and neutral metabolites (**43–56**) have been isolated and their structures elucidated. In analogy to retinoic acid, the tetraene side chain was shortened resulting in C_{16}-, C_{14}-, and C_{11}-skeletons. The aromatic moiety remained intact, in some compounds, however, the methoxy group was cleaved, yielding a phenolic end group. Carboxylic acids and their conjugates, respectively, lactones and hydroxylated lactones, have been identified.

Synthesis of some urinary metabolites of Ro 10-9359

The synthesis of the urinary metabolites **43**, **46** and **48** of Ro 10-9359 was recently completed[123] and is outlined briefly in figure 22. The intermediate aromatic ketone **21** is first reacted with the Grignard reagent of the bromoketal **57** yielding the C_{16}-ketal **58**. Subsequent treatment with strong acid furnishes the lactol **59** which is readily oxidized to the corresponding lactone, the urinary metabolite **48**. This compound can then be readily transformed into the metabolites **43** and **46** by heating with pyridine hydrochloride at 190 °C. Cleavage at 160 °C leads to the corresponding methoxy acid **60**. It is worthwhile to mention here that by this sequence of reactions it is possible to insert a CH_2-group between the unsaturation and the polar end group, a situation which does not occur very often in polyene chemistry.

Mechanism of action of retinoids

The molecular mechanism by which retinoids control growth and differentiation of epithelial tissues as well as the mechanism of their carcinostatic ability is still unknown. There is no doubt, however, that the regu-

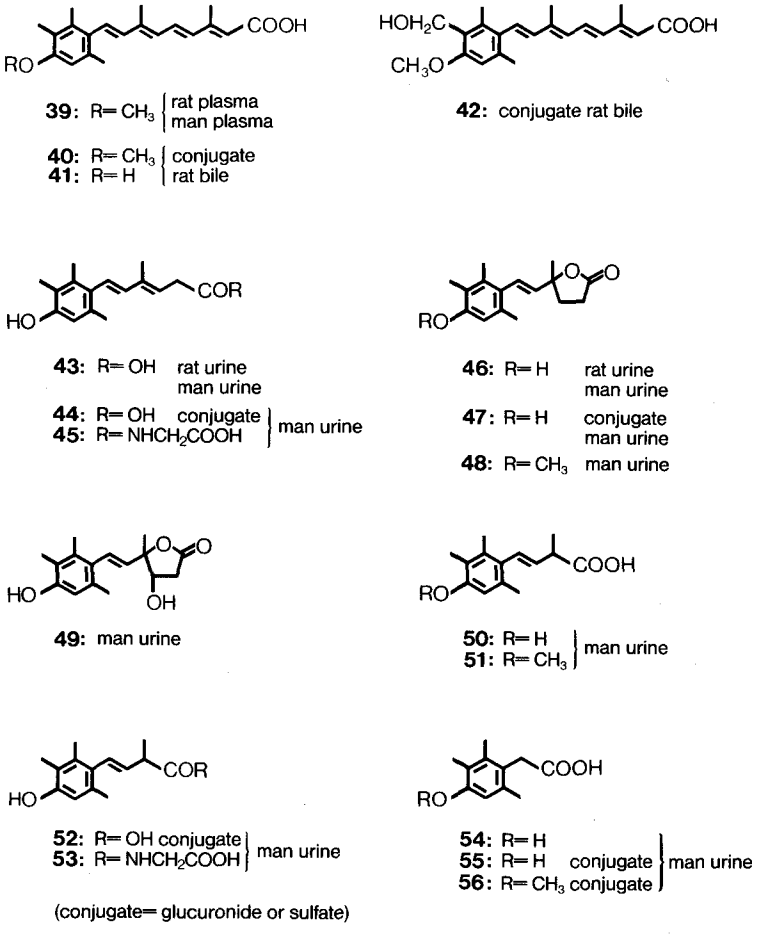


Fig. 21. Metabolites of the aromatic retinoid Ro 10-9359.

lation of epithelial differentiation is one of the most important properties of this class of compounds. Dermatological disorders, such as ichthyosis or psoriasis show increased and/or qualitatively changed epidermal keratinization. Retinoids, e.g. retinoic acid, inhibit normal as well as pathological keratinization and may, therefore, have a favorable influence on these skin disorders[124,125]. Considering the prophylaxis of precancerous and cancerous conditions, retinoids may influence the transformation process from a normal to a neoplastic tissue. In fact, in organ cultures, retinoids prevent and counteract hyperplastic and metaplastic changes of tracheal and prostate epithelium induced by vitamin A-deficiency or by carcinogens[59,90,91,94,126].

With respect to the therapeutic effect of retinoids on established benign and malignant epithelial tumors, recent investigations have been carried out in our laboratories which may have some relevance to the mechanism of action of retinoids in general. Chemically induced papillomas in mice have been treated with the aromatic retinoid Ro 10-9359. By autoradiographic and histopathological studies it has been shown that under this treatment the number of DNA-synthesizing cells, as measured by the labelling index and the length of the cell cycle, was not affected. Thus, the regression of these tumors is not a consequence of an inhibition of the proliferation activity. In addition, the histometrical method revealed that the reduction of the size of papillomas was mainly due to a loss of horn and cells, as manifested by necroses, these being 3-10 times larger than in nontreated papillomas[127]. An attempt was made to elucidate the underlying mechanism of necrosis by means of electronmicroscopic investigations[128]. When treating chemically induced papillomas with Ro 10-9359, the following phenomena have been observed, as illustrated in figure 23.

1. Smooth vesicles are extruded into the intercellular space by a process of budding and pinching-off of the plasma membrane. 2. Intracellularly many coated vesicles are formed in the region of the Golgi apparatus. These vesicles approach the plasma membrane, then fuse with it and secrete a homogenous substance into the intercellular space. It is highly probable that the coated vesicles contain glycoproteins and/or mucopolysaccharides which are secreted into and fill out the intercellular space. It is well-known from biochemical investigations that vitamin A and other retinoids have a stimulating influence on the synthesis of mucopolysaccharides and glycoproteins[129,130]. 3. In electronmicroscopic sections a reduction of tonofilaments and desmosomes was observed. In conclusion, a dilatation of the intercellular space is taking place, which may lead to a separation and disruption of cell membrane contacts and cell anchorage leading to cell necrosis and, consequently, to cell loss.

Another possible action of retinoids may consist in their immunological effects. Thus, it has been found that retinoids accelerate rejection of skin grafts[131,132] and also show an immune-mediated effect in several tumor systems[133-136].

A most interesting new aspect of the mechanism of action of retinoids is presented by the recent detection of a cellular retinoic acid-binding protein. This protein may well be a prerequisite for the action of retinoids on certain normal and tumor cells[137-140].

Clinical results with aromatic retinoids

As already mentioned, positive clinical results have been obtained by the treatment with retinoic acid of

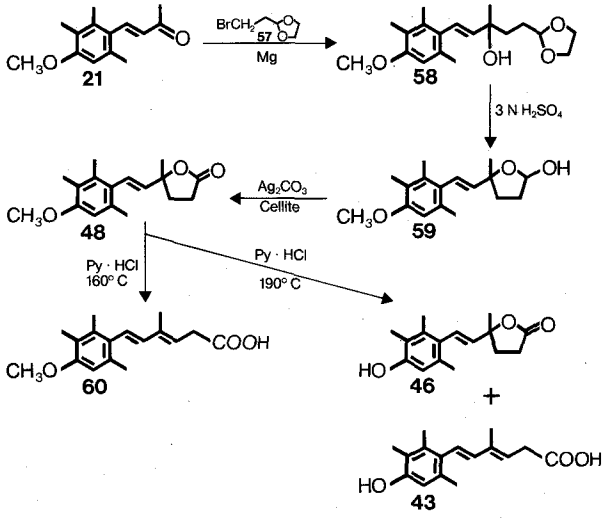


Fig. 22. Synthesis of some urinary metabolites of Ro 10-9359.

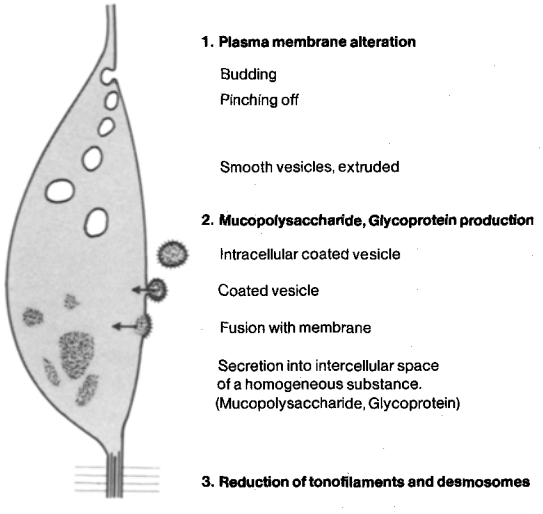


Fig. 23. Morphological effects on papilloma cells induced by the aromatic retinoid Ro 10-9359.

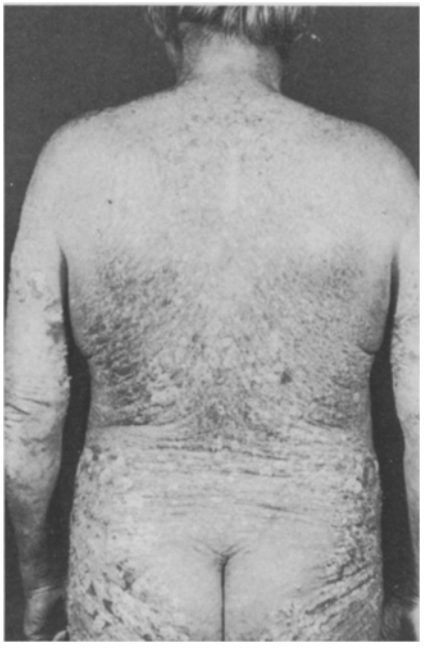


Fig. 24. Patient before treatment.

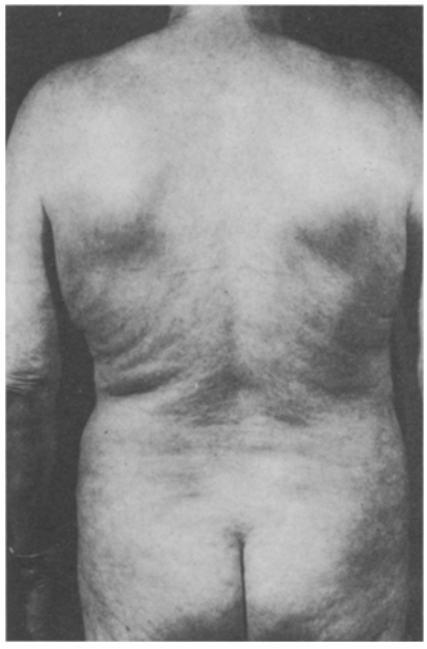


Fig. 25. The same patient after a 6-week oral treatment with 50–100 mg of Ro 10-9359 daily.

actinic keratoses, basal cell carcinomas and papillomas of the urinary bladder. New prospects have now been opened up by the treatment of various dermatological disorders with aromatic retinoids. Thus, in patients with severe acne vulgaris, good results with less skin irritation than with retinoic acid have been obtained with the retinoid Ro 11-1430[141]. The aromatic retinoid Ro 10-9359 has been shown to be a most successful drug for the oral therapy of keratinizing dermatoses and particularly of psoriasis, including the severest cases, such as generalized erythrodermic and pustulous forms[142-145]. Figures 24 and 25 demonstrate the successful treatment of a patient suffering from generalized psoriasis spread over more than 75% of the skin. Furthermore, in preliminary clinical trials, several forms of precancerous conditions could be successfully treated with oral Ro 10-9359. Thus, partial or even complete remissions have been obtained in patients with actinic keratoses[145], leukoplakias of the oral cavity[146], hyperkeratosis of the larynx[147], or papillomas of the urinary bladder[148].

Concluding remarks

In this article we have reviewed some recent investigations in the retinoid field. The retinoids, structural analogs of vitamin A and retinoic acid, represent a new class of compounds with remarkable prophylactic and therapeutic activities in oncology and dermatology. Based on experience gained with vitamin A and carotenoids, a large series of retinoids has been synthesized. Well-established reaction sequences have been developed, and the most important compounds are now readily available on a technical scale.

The aromatic retinoids Ro 10-9359 and Ro 11-1430 proved to be especially interesting. In animal experiments with chemically induced papillomas and carcinomas, these compounds have been found to be 10 times more active than retinoic acid. Successful clinical results have been obtained with patients suffering from acne vulgaris, psoriasis, actinic keratosis, leukoplakia of the oral cavity, hyperkeratosis of the larynx or papilloma of the urinary bladder. Attempts have been made to elucidate the unique mode of action of retinoids which differs fundamentally from that of today's cytostatic agents. It is hoped that these investigations will help us to develop new methods of prevention and therapy of preneoplastic and neoplastic epithelial lesions.

With the aid of our animal model and on the basis of the definition of the therapeutic ratio, we hope to be able to dissociate the antitumor effect and the hypervitaminosis A syndrome still further. It remains to the organic chemist to design and synthesize further structural variations of the retinoid skeleton that will be superior to the compounds used today in the chemotherapy of cancer.

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Attraction of *Scolytus scolytus* (F.) to the components of Multilure, the aggregation pheromone of *S. multistriatus* (Marsham) (Coleoptera: Scolytidae)[1]

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Summary. The attraction of *S. scolytus* to the components of Multilure is described. 4-Methyl-3-heptanol is an attractant which is synergised by α-cubebene. Multistriatin appears to be an inhibitor. A combination of 4-methyl-3-heptanol and cubeb oil is more effective as a bait for *S. scolytus* than Multilure.

In the United Kingdom a major vector of *Ceratocystis ulmi* (Buism.) C. Moreau, the causal fungus of Dutch elm disease, is the larger European elm bark beetle, *Scolytus scolytus*. When unmated beetles bore into English elm, *Ulmus procera* Salis., the volatiles produced include threo and erythro 4-methyl-3-heptanol **I**, α-multistriatin **II** and α-cubebene **III**[3,4]. (-)-Threo-I, (-)-II and (-)-III have been identified as components of the aggregation pheromone of *S. multistriatus*[5-7]. In both species **I** and **II** are beetle-associated whilst **III** is a host metabolite.

In *S. scolytus* the male produces the secondary attractant[8]. There is also a differential production of **I** and **II** between the sexes. α-Multistriatin is produced mainly by females whilst males produce 4-methyl-3-heptanol[4]. This is in contrast to *S. multistriatus* where the components, **I** and **II**, of the aggregation pheromone are produced by the female[5].
A mixture, Multilure, of synthetic isomers of 4-methyl-3-heptanol and multistriatin, and distilled cubeb oil (70% α-cubebene) is effective in trapping large numbers of *S. multistriatus* in the USA[9], but does not appear to attract large