Tetrahedron Vol. 35, pp. 2843 to 2853.
Pergamon Press Ltd., 1979. Printed in Great Britain.

TETRAHEDRON REPORT NUMBER 70

THE PIGMENTS OF FLY AGARIC, *AMANITA MUSCARIA*

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(*Received for publication in revised form:* 19 *February* 1979)

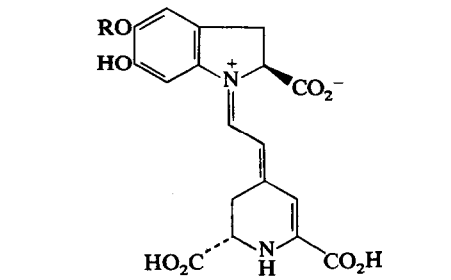
Abstract—The history of the investigation of the pigments of the toadstool *Amanita muscaria* is reviewed. Earlier studies are shown to be incorrect and now the pigments are demonstrated to be structurally related to the betalains (**2** and **3**). The elucidation of the constitutions of yellow muscaflavin (**12a**), the orange muscaurins (Scheme 3), and purple muscapurpurin (Scheme 4) are discussed. Biogenetic proposals based upon structural correlations between the betalains and muscaflavin, the muscaurins, and muscapurpurin are put forward.

The investigation of natural colouring matters is a field of study which has always fascinated organic chemists. Many enquiries have extended over long periods and they make interesting contributions to the history of the development of the subject.[1] These include the classic investigations[2] by Willstätter and Robinson of the anthocyanins (1915–1928), the lengthy enquiry (1835–1961) to determine the structure of the orcein and litmus pigments,[3] and the investigation of chlorophyll-*a* initiated by Berzelius in 1838 and dominated by Woodward's total synthesis which was completed in 1960.[4]

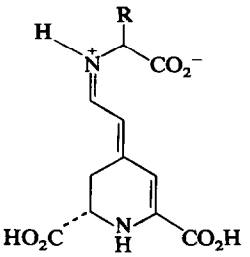
The examination of the so-called "nitrogeneous anthocyanins"[5] is also representative of this type of investigation. The scrutiny of this class of natural pigments extends from 1876 when they were first isolated by Bischoff to the initial structural studies which led to the belief that these compounds were nitrogen-containing flavylium derivatives.[5] These opinions were totally changed when it became clear that betanin, isolated from the red beet, *Beta vulgaris*, belonged to a new class of natural products now regarded as being phytochemically characteristic of the Centrospermae.[6] By the heroic investigations of Dreiding,[6,7] Mabry,[7-9] and Piattelli,[10] these pigments were eventually recognised as the betalain class of natural pigments.[7] The betalain pigments are amino-acid condensation products derived from betalamic acid (**4a**). These pigments are divided into two classes, the red-violet betacyanins and the orange-yellow betaxanthins. The betacyanins are glycosides (R = carbohydrate residue) of betanidin (**1**; R = H) and betanidin is a condensation product of cyclodopa and betalamic acid (**4a**). The betaxanthins (**3**) are also condensation products of betalamic acid (**4a**), but in this case the rest of their structure is provided by natural aliphatic α-amino acids.

The betacyanins (**2**) and betaxanthins (**3**) are of considerable phylogenetic significance: their primary association with ten families belonging to the order Centrospermae has been noted.[6-10] their isolation from the beet, *Beta vulgaris* (*Chenopodiaceae*), is well known, but the discovery[6,10,11] of the phytochemical association of betalains with cacti (*Cactaceae*) and other succulent plants (*Aizoaceae*) is not so generally recognised.

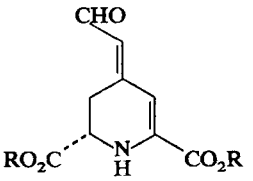
Succulent plants including cacti are characteristic of the desert, so our discovery that the toadstool, *Amanita muscaria*, found in the damp forests, also produced a whole range of pigments related to the betacyanins (**2**) and betaxanthins (**3**) was totally unexpected. Our results[12-14] are quite different from those reported by Kögl and Erxleben[15] in 1930 for their chemical examination of this toadstool.



1 R = H Betanidin
2 R = Carbohydrate residue Betacyanins



3 Betaxanthins



4a R = H Betalamic acid
4b R = Me

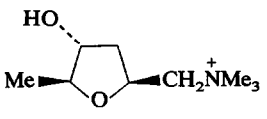
The toadstool fly agaric, *Amanita muscaria* (L. ex Fries) Hooker has long been a source of interest and fascination. It is widely distributed throughout the forests of Europe, Asia, and North America, and its striking appearance is well known. An excellent colour photograph of the toadstool appears in a comprehensive review of the extractives of *Amanita muscaria* published by Eugster.[16] The toadstools are frequently depicted with bright red, white-spotted caps in children's story books. On New Year's Eve in German pastry shops one can buy Glückspilze: these are imitations of fly agaric made from marzipan and chocolate. They are given to friends as good luck presents, but it is not clear how the gift of imitations of poisonous mushrooms came to be regarded as an act of friendship!

Much folklore is associated with fly agaric. There are many reports[17,18] of its use in endemic medicine and in religious ceremonies practised by nomadic tribes in Asia and Asia Minor. In small quantities fly agaric is claimed to cause hallucinations followed by narcosis. The toadstool has also been regarded as a symbol of fertility: this may well be associated with its striking appearance when the fruiting bodies sprout from the earth and unfold.

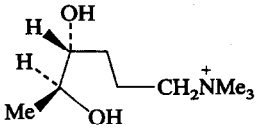
Amanita muscaria has provided two main challenges to the natural products chemist: (i) the investigation of the compounds responsible for its psycho-activity and (ii) the isolation and elucidation of the constitution of the pigments responsible for the striking red colour of the cap of the toadstool. We now review progress in the investigation of physiologically active compounds and then discuss our own work on the pigments.

Psycho-active compounds from Amanita muscaria

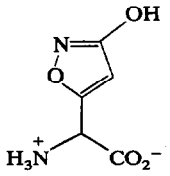
These studies have been excellently reviewed by Eugster.[16] It is therefore necessary only to mention the isolation of the alkaloids muscarine (**5**) and muscaridine (**6**) and the unusual α-amino acid ibotenic acid (**7**).[19]



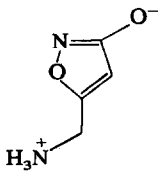
5 Muscarine



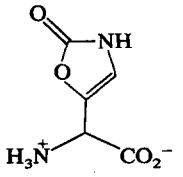
6 Muscaridine



7 Ibotenic acid



8 Muscimol



9 Muscazone

Ibotenic acid (**7**) undergoes easy decarboxylation yielding muscimol (**8**) even in neutral solution. Ibotenic acid (**7**) is transformed into its photoisomer, muscazone (**9**) by irradiation.

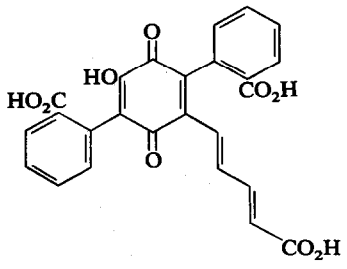
Ibotenic acid has insecticidal activity against houseflies and it has also been isolated from *Amanita strobiliformis* and *Amanita pantherina*.[19]

Pigments of Amanita muscaria

In 1930, Kögl and Erxleben reported the isolation, from the red skin of fly agaric, of a red, crystalline compound which they called muscarufin.[15] The quinonoid structure (**10**) which they proposed for this compound became widely accepted, particularly as a result of its publicity in many textbooks, monographs, and reviews.

The development of methods of microanalysis by Pregl, who was awarded the Nobel Prize in Chemistry in 1923, has revolutionised natural product chemistry since that time. It then became possible for structural investigations to be carried out on very small quantities of material and the microdegradations on muscarufin and its derivatives which were reported[15] were widely regarded as outstanding examples of these new and powerful techniques. However, the evidence and arguments presented in support of the structural proposal (**10**) for muscarufin[15] have not stood the test of time. Furthermore, attempts by synthesis to provide evidence in favour of the structure (**10**) have not been rewarded.[20,21] Attempts by several research groups to repeat the isolation of muscarufin following Kögl's procedure have failed.[22] In particular, isolation of muscarufin *via* its silver salt was claimed.[15] However, we found that, although the pigment is precipitated by silver ions, the coloured material cannot be recovered unchanged.

We now report our results on the characterisation of the pigments isolated from 300 kg of the caps of *Amanita muscaria* which were collected in the Black Forest during the autumn of 1970. The red skins (20 kg) peeled from the caps were extracted with cold methanol. Extremely careful evaporation and lyophilisation yielded a dark brown powder (600 g) which could be stored at −40° without spectroscopic change (λ_{max} 475 nm). This characteristic absorption (λ_{max} 475 nm) was used to monitor the stability of the pigments and the efficiency of methods of purification and fractionation. It was found that the intensity of absorption of the crude pigment in methanolic solution decreased by 10–15% during one day at room temperature, acidic conditions destroyed the pigment during minutes and basic conditions during

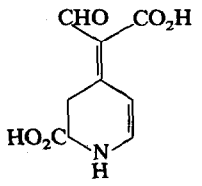


10 Muscarufin

seconds. The pigments could not be extracted from aqueous solution with organic solvents.

Ultimately it was found[12,13] that fractionation could only be achieved using a sequence of chromatography on Sephadex columns [(i) CM-Sephadex C-25; (ii) Sephadex G-10; (iii) DEAE-Sephadex A-25 elution with an increasing (0.3 to 1.0 M) sodium chloride solution gradient; (iv) desalting on Sephadex G-10]. In order to avoid decomposition of the pigments, steps (iii) and (iv) had to be carried out rapidly, in the dark, at 5 to 3°C with small amounts of material (5–50 mg). Only by observing these precautions could reproducible and satisfactory results be obtained. Step (iii) yielded nine main fractions which by repeated chromatography gave amorphous sodium salts with maximal extinction coefficients. The yellow sodium salt (λ_{max} 420 nm) was named muscaflavin, the seven orange salts (λ_{max} 475 nm) were named muscaurins I–VII, and the purple salt (λ_{max} 303 and 540 nm) muscapurpurin. All these pigments were optically active and contained nitrogen (~5%). Clearly they were not structurally related to the quinonoid formulation proposed for muscarufin (**10**). In fact, we have never succeeded in isolating any substance exhibiting the properties reported for muscarufin.

Muscaflavin (**12a**). Treatment of the sodium salt of muscaflavin with methanolic hydrogen chloride gave muscaflavin dimethyl ester which was obtained as a crystalline derivative, $C_{11}H_{13}NO_5$. This dimethyl ester could also be obtained by treatment of the disodium salt, first with acid and then with diazomethane.



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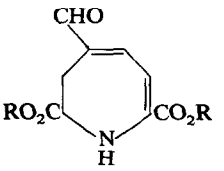
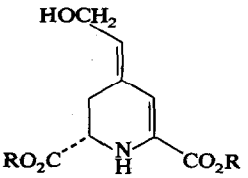
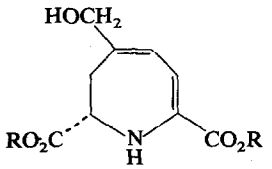


12a R = H Muscaflavin
12 R = Me



13a R = H
13b R = CH_3



14a R = H
14b R = Me

At this point in our investigations, it was recognised (see following section) that there was a remarkable similarity between the ultraviolet and visible spectra of the muscaurins and the betalains. Furthermore, betalamic acid (**4a**)[23–25] and muscaflavin were isomers, $C_9H_9NO_5$. Initially the constitutional formula (**11**) was proposed by us for muscaflavin,[14,26] but comparison of the electronic spectra (Fig. 1) of muscaflavin and betalamic acid clearly showed an overall correspondence, although

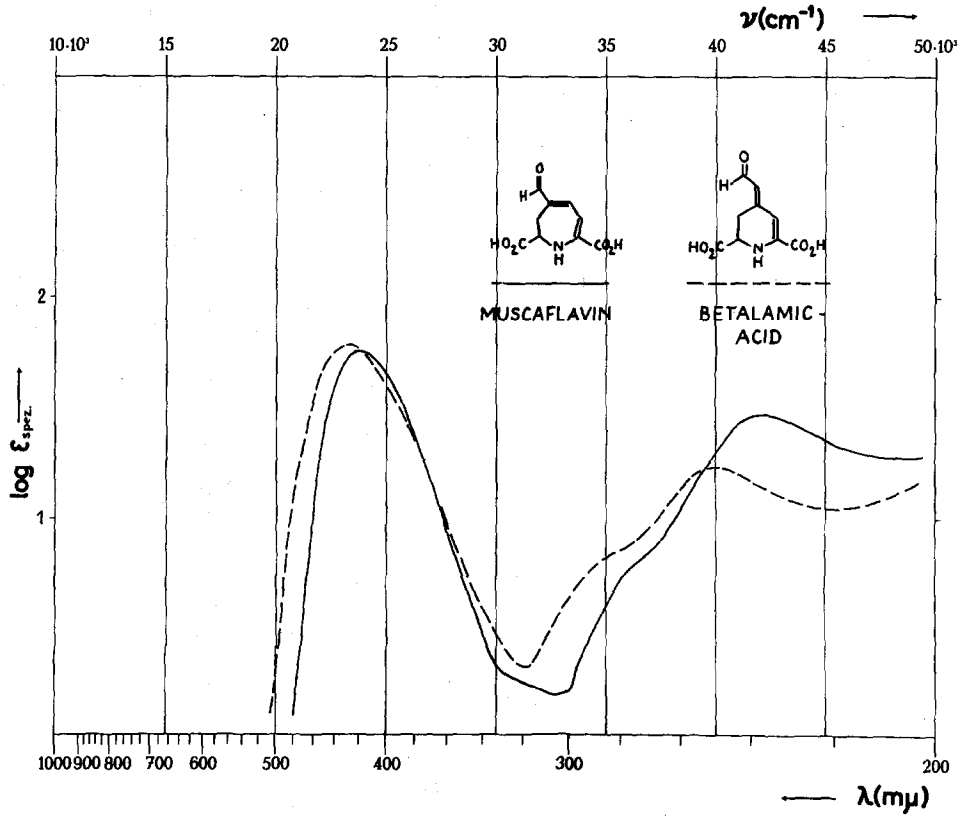
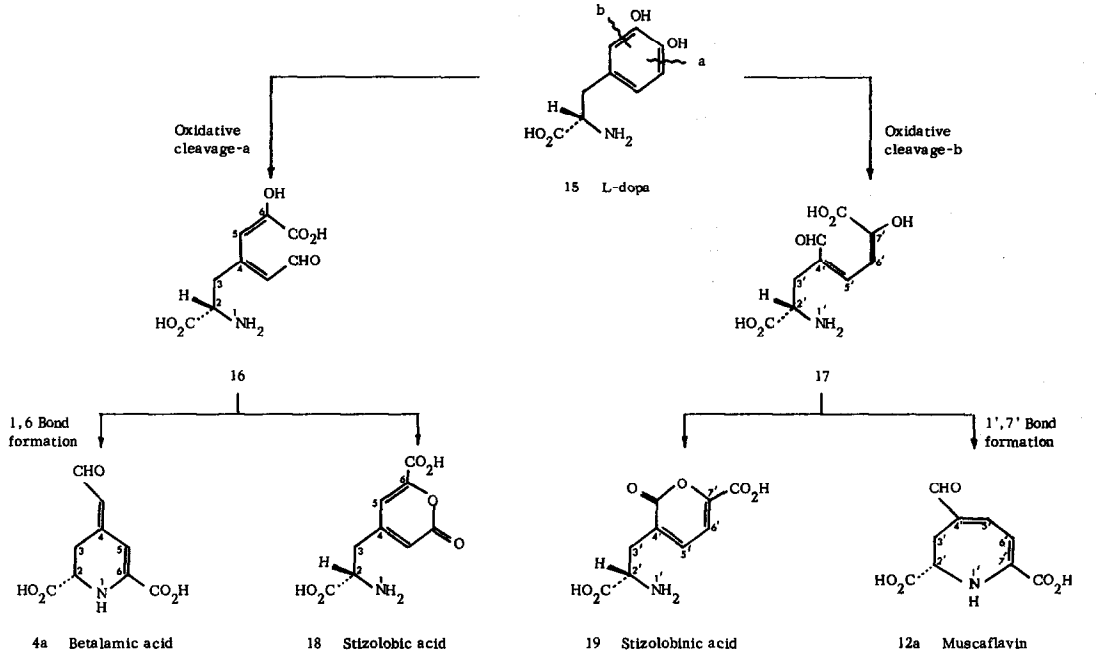


Fig. 1



Scheme 1. Biogenetic proposal for structural correlation between betalamic acid (**4a**), muscaflavin (**12a**), stizolobic acid (**18**) and stizolobinic acid (**19**).

distinctive differences were apparent, particularly at shorter wavelengths. Sodium borohydride reduction of dimethyl betalamate (**4b**) yielded the dimethyl ester (**13b**): muscaflavin dimethyl ester similarly yielded dihydromuscaflavin dimethyl ester. Detailed comparison of the proton-proton coupling constants in the ^{1}H NMR spectra of the dimethyl esters of betalamic acid (**4b**), muscaflavin (**12b**), dihydrobetalamic acid (**13b**), and dihydromuscaflavin (**14b**) demonstrated with certainty that muscaflavin did not have the constitution (**11**). Muscaflavin was therefore formulated as a dihydroazepine derivative (**12a**).[27] This was a novel structure for a natural product, but reassuring support was obtained by considering a biogenetic proposal (Scheme 1) based upon the established biosynthetic pathway to betalamic acid (**4a**).[28,29]

It has been established,[28,29] using ^{14}C- and ^{3}H-labelled precursors, that betalamic acid (**4a**) is formed in higher plants from L-tyrosine and L-3,4-dihydroxyphenylalanine (**15**; L-dopa). The transformation may be represented (Scheme 1) as involving the well-known oxidative cleavage of the catechol residue at bond-a (see **15**). This gives the intermediate (**16**) which can then cyclise *via* a carbinolamine derived by interaction between the amino group in position 1 and the carbonyl group in position 6. This intermediate (**16**) can also cyclise *via* an intermediate lactol which can then be further transformed oxidatively to give the known α-amino acid, stizolobic acid (**18**).[30]

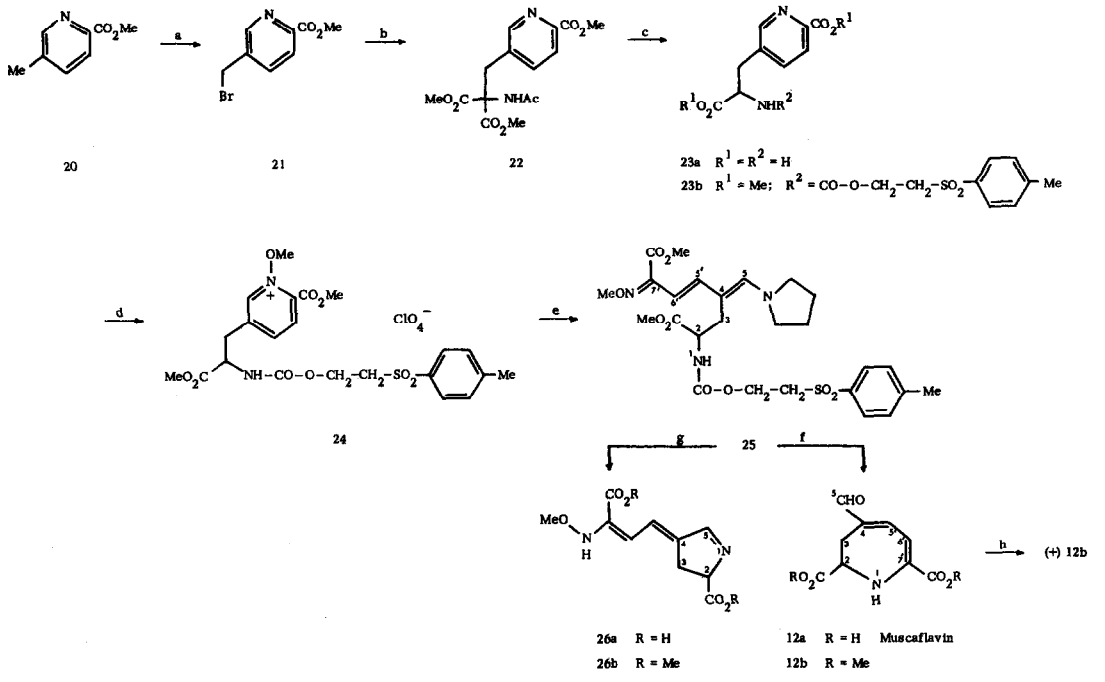
Alternatively, oxidative cleavage of L-dopa (**15**) at bond-b could give rise to the intermediate (**17**) which by 1'–7' bond formation could provide an acceptable biosynthetic pathway to muscaflavin (**12a**). The intermediate (**17**) is also an acceptable biosynthetic precursor of stizolobinic acid (**19**).

These proposals (Scheme 1) not only provided support for the proposed constitution of muscaflavin (**12a**) but were also acceptable in giving reasonable biosynthetic routes[30] to stizolobic acid (**18**) and stizolobinic acid (**19**). These two unusual α-amino acids were first isolated from the tropical bean, *Stizolobium hassjo*.[30] Subsequently, these two α-amino acids were both isolated from *Amanita pantharina*[31] and from *Amanita muscaria*. Recently the general features of the biosynthetic pathways to stizolobic acid (**18**) and stizolobinic acid (**19**) proposed in Scheme 1 have been confirmed by Komamine and his collaborators,[32] who have studied the incorporation of labelled precursors using *Stizolobium hassjo* as well as *Amanita pantharina*.

This congeneric association in *Amanita muscaria* of stizolobic acid (**18**) and stizolobinic acid (**19**) as well as muscaflavin (**12a**) indicates that the proposals in Scheme 1 are well based. Additional sources of support have been derived from our investigation of the constitutions of the muscaurins (see following section).

The biogenetic proposal (Scheme 1) for muscaflavin (**12a**) provided a basis for our conception of a biomimetic synthesis of this compound (Scheme 2).[33] The key step in the planned synthesis was the generation of the proposed intermediate (**17**), or its synthetic equivalent (*cf* **25**), by nucleophilic attack by hydroxide anion upon an appropriately substituted pyridinium cation (**24**). The base catalysed ring cleavage of N-methoxypyridinium cations was first described by Eisenthal and Katritzky.[34]

The synthesis of muscaflavin dimethyl ester (**12b**) is summarised in Scheme 2. Bromination of 2-methoxycarbonyl-5-methylpyridine (**20**) with N-bromosuccinimide yielded the bromomethyl derivative (**21**) which was used to alkylate dimethyl



Reagents: a = N-bromosuccinimide; b = $AcNHCH(CO_2Me)_2$; c (i) HBr/H_2O (ii) MeOH/HCl (iii) p-$MeC_6H_4SO_2CH_2CH_2OCOCl$; d = (i) m-$ClC_6H_4CO_3H/CH_2Cl_2$ (ii) Me_2SO_4/MeCN (iii) $NaClO_4$; e = pyrrolidine/tetrahydrofuran/0°; f = (i) 0.5M aqueous potassium hydroxide (ii) HBr/HCO_2H (iii) diazomethane; g = (i) $HClO_4/HCO_2H$ (ii) CH_2N_2; h = potato starch.

Scheme 2. Synthesis of (+)muscaflavin dimethyl ester

acetamidomalonate. The derived acetamidomalonic ester (**22**) was hydrolysed and decarboxylated by heating with aqueous hydrobromic acid yielding the amino acid (**23a**) which was esterified and N-protected giving the derivative (**23b**). Oxidation with *m*-chloroperbenzoic acid gave the corresponding pyridine N-oxide which was O-methylated with dimethyl sulphate followed by treatment with sodium perchlorate, thus yielding the N-methoxypyridinium perchlorate (**24**). Base catalysed cleavage of the salt (**24**) was observed but this reaction proceeded in low yield: the major reactions were deprotonation with loss of formaldehyde from the methoxyl group,[35] plus base catalysed removal of the N-protecting group, giving the amino acid (**23a**). A much more encouraging result was obtained by treatment of the intermediate (**24**) with pyrrolidine at 0°. This reaction yielded the enamine (**25**) in which the E-configuration of the 5′–6′ ethylenic bond was established by the examination of nuclear Overhauser effects. Base catalysed removal of the N-protecting group of the enamine (**25**) followed by acid catalysed cyclisation with hydrobromic acid in formic acid and treatment with diazomethane gave a mixture of the esters (**26b** and **12b**). Unfortunately by this sequence the yield of the required ester (**12b**) was very low and the major product was the isomer (**26b**). However, it was found that the acid catalysed transformation (**26b** → **12b**) could be achieved in good yield using perchloric acid in formic acid. The synthetic dimethyl ester (**12b**) was identical with the racemate obtained by heating the (+)-dimethyl ester derived from natural muscaflavin. Finally, the resolution of the synthetic (±)-dimethyl ester (**12b**) has been successfully achieved by chromatography on potato starch in 1M-potassium citrate buffer at pH 7.0.[36] The (±)-amino acid **23a** and its N-oxide have been similarly resolved.[36]

Muscaurins I—VII. The use of Sephadex chromatography leading to the isolation of seven major fractions has been described. Although it became clear that some of these fractions were indeed mixtures, it was nevertheless possible to recognise that the muscaurins all belonged to the same class of natural product. The best analytical method for the muscaurins was found to be high voltage electrophoresis[11] and all these orange pigments showed anionic movement down to pH 2.

The progress of structural studies on muscaflavin coupled with consideration of the biogenetic correlations given in Scheme 1, provided a hint that the muscaurins and the betaxanthin pigments (**3**) might belong to closely related structural types. Agreement between the visible spectra (Fig. 2) of muscaurin I, muscaurin II, and a typical betalain pigment, vulgaxanthin II, left no doubt that the same chromophore was common to these three natural products.

A remarkable step forward was made when it was shown that acidic hydrolysis of the muscaurins gave α-amino acids and betalamic acid (**4a**). Esterification of the hydrolysate, followed by extraction gave dimethyl betalamate (**4b**) which was fully characterised and was shown to be identical with authentic material. After extraction of the dimethyl

Table. Amino acids obtained by the hydrolysis of muscaurins I–VII

Muscaurin	Hydrolysis products
I	Ibotenic acid (**7**)
II	Stizolobic acid (**18**)
III	Glutamic acid mainly + α-amino adipic acid, and aspartic acid
IV	Aspartic acid mainly + glutamic acid
V	Glutamine, leucine, valine, proline, and asparagine
VI	Glutamine and proline
VII	Histidine

betalamate (**4b**), the α-amino acids were detected by electrophoresis and paper chromatography. They were then transformed into their volatile N-trifluoracetyl methyl esters and identified by gas liquid chromatography—mass spectrometry. The α-amino acids identified in the hydrolysates are summarised in the Table.

From the information given in the Table, it was clear that the muscaurins I, II, and VII behaved as single compounds, whereas muscaurins III, IV, V, and VI behaved as mixtures. Hydrolysis of muscaurins III and IV gave mixtures of acidic α-amino acids, whereas muscaurins V and VI gave mixtures of neutral amino acids. This explained the difference in electrophoretic behaviour between muscaurins III and IV on the one hand and muscaurins V and VI on the other.

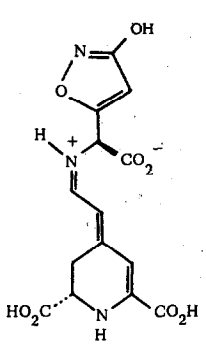
All the muscaurins have the same chromophore (Fig. 2, λ_{max} 475 nm) as the betaxanthins (**3**) and it was clear that the muscaurins are either identical with known betaxanthins (**3**) or are new betaxanthins in which the betalamic acid residue is associated with different α-amino acids. On the basis of the information given in the Table, the muscaurins present in the red skins of the caps of *Amanita muscaria* are formulated as the betaxanthins (**27–37**) (Scheme 3).

As is indicated in Scheme 3, the compounds **29, 31, 32,** and **35** are identical with vulgaxanthin I,[37] miraxanthin III,[38] vulgaxanthin II,[37] and indicaxanthin[39] respectively. Vulgaxanthin I (**29**) and vulgaxanthin II (**32**) have been previously isolated from the red beet, *Beta vulgaris*, miraxanthin III (**31**) from *Mirabilis jalapa*, and indicaxanthin (**35**) from the fruit of the cactus, *Opuntia ficus-indica*. One of the most interesting rewards of the investigation of the pigments of fly agaric is the discovery of muscaurin I (**27**), muscaurin II (**28**), and muscaurin VII (**37**). Muscaurin I contains a residue derived from ibotenic acid (**7**) and muscaurin II is similarly related to stizolobic acid (**18**).[40] In fact, the isolation of muscaurin II (**28**) should be regarded as a persuasive vindication of the biogenetic proposals presented in Scheme 1.

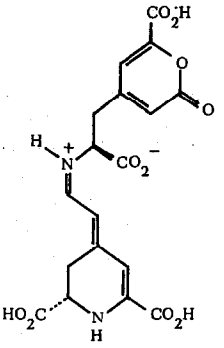
The structures (**27–37**) have been confirmed by synthesis.[40] Betanin (**2**; R = β-D-glucopyranosyl) was hydrolysed with ammonia in the presence of a 5- to 10-fold excess of the appropriate amino acid. This established an equilibration reaction yielding the required muscaurin. The progress of the reaction was monitored spectroscopically and when the decrease in the maximal absorption due to betanin (λ_{max} 540 nm) and the increase in absorption due to the muscaurin (λ_{max} 475 nm) was complete then the reaction mixture was fractionated by Sephadex chromatography. This yielded synthetic muscaurins (**27–37**). By a combination of analytical chromatography (Sephadex), electrophoresis and electronic spectral determination, the synthetic muscaurins (with one exception) were shown to be identical with the natural pigments. The exception was muscaurin II (**28**). The natural product presumably has the absolute configuration (**28**), whereas the reaction between betanin (**2**) and racemic stizolobic acid would be expected to yield a mixture of two diastereomers.[40] These two compounds have not yet been separated, but with this reservation concerning muscaurin II (**28**), our synthetic studies may be regarded as a confirmation of the different constitutions of the muscaurins (Scheme 3). This claim of their formal total synthesis of course relies upon the elegant total syntheses of betalamic acid (**4a**) and the betalains achieved first by Hermann and Dreiding[41] and recently in a very different approach by Büchi, Fliri and Shapiro.[42]

Muscapurpurin (Scheme 4). In the preceding section describing the fractionation of the pigments of *Amanita muscaria*, the isolation of a purple pigment, muscapurpurin, is mentioned. In contrast with the muscaurins (Scheme 3) which exhibit the highly characteristic betaxanthin (**3**) chromophore (λ_{max} 475 nm; Fig. 2); muscapurpurin shows absorption at a longer wavelength (λ_{max} 540 nm). This indication of more extended conjugation suggested that muscapurpurin possessed a similar chromophore (Fig. 3) to that of betanin (**2**; R = β-D-glucopyranosyl). In addition, it was found that the condensation of betalamic acid (**4a**) with aromatic amines gave products[43] which were good models for the chromophore of betacyanins (**2**) and muscapurpurin.

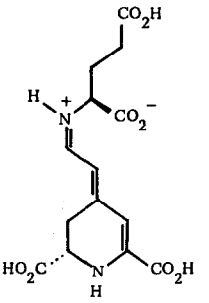
Acidic hydrolysis of muscapurpurin gave a product which did not give a normal colouration with ninhydrin characteristic of α-amino acids. Furthermore, the hydrolysis product could not be recognised as a known α-amino acid either by its electrophoretic behaviour or by paper chromatography. However, N-trifluoracetylation and reaction with diazomethane gave an N-trifluoracetyl methyl ester which exhibited the following mass spectral fragmentation pattern (*m/e* 349, 321, 290, 262, 236, 230). These ions showed an informative regular difference of two atomic mass units when compared with the mass spectral fragmentation pattern of the N-trifluoracetyl methyl ester of stizolobic acid (*m/e* 351, 323, 292, 264, 238, and 232). On the basis of this evidence,[43] the interesting possibility was considered that muscapurpurin could have one of four possible structures (**38, 39, 40,** or **41**). These four structures (Scheme 4) were essentially delineated by two biogenetic programmes for their formation. The first proposal (Scheme 5) is based upon the possibility that the dehydroamino acid residue in muscapurpurin arises either from stizolobic acid (**18**) or from stizolobinic acid (**19**). In either case the process involves β-addition of the amino group to the α-pyrone-carboxylic acid residue followed by oxidative dehydrogenation.



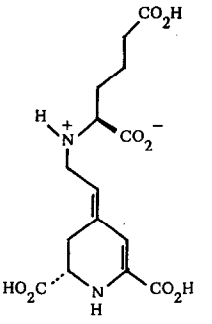
27 Muscaurin I



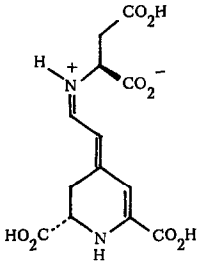
28 Muscaurin II



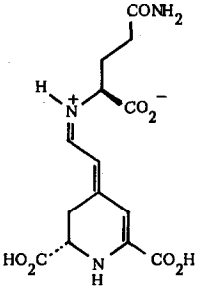
29 Vulgaxanthin I[a,b]



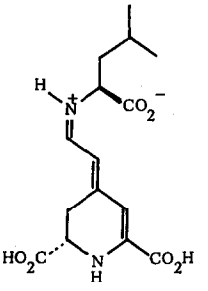
30[a]



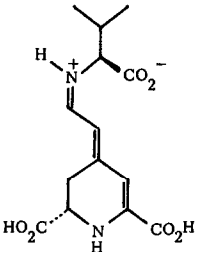
31 Miraxanthin III[a,b]



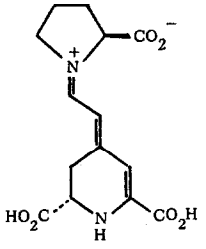
32 Vulgaxanthin II[c,d]



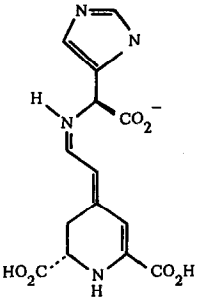
33[c]



34[c]



35 Indicaxanthin[c,d]



37 Muscaurin VII

[a] Betaxanthin present in muscaurin III

[b] Betaxanthin present in muscaurin IV

[c] Betaxanthin present in muscaurin V

[d] Betaxanthin present in muscaurin VI

Scheme 3. Muscaurins present in *Amanita muscaria*

An alternative biosynthetic proposal (Scheme 6) for the formation of muscapurpurin can be envisaged. This is based upon the proposition that just as cyclodopa (**42**) is believed to be involved[28,29] in the biosynthesis of betanidin (**1**) and the betacyanins (**2**), so the oxidative cleavage of cyclodopa (Scheme 6) could give rise to the dehydro-amino acid component of the muscapurpurin structure.

It is perhaps unwise to consider at this stage the relative merits of the four possible constitutions of muscapurpurin (Scheme 4) or the relative appeal of the two biogenetic proposals (Scheme 5 or Scheme 6). However, subject to this note of caution, it may

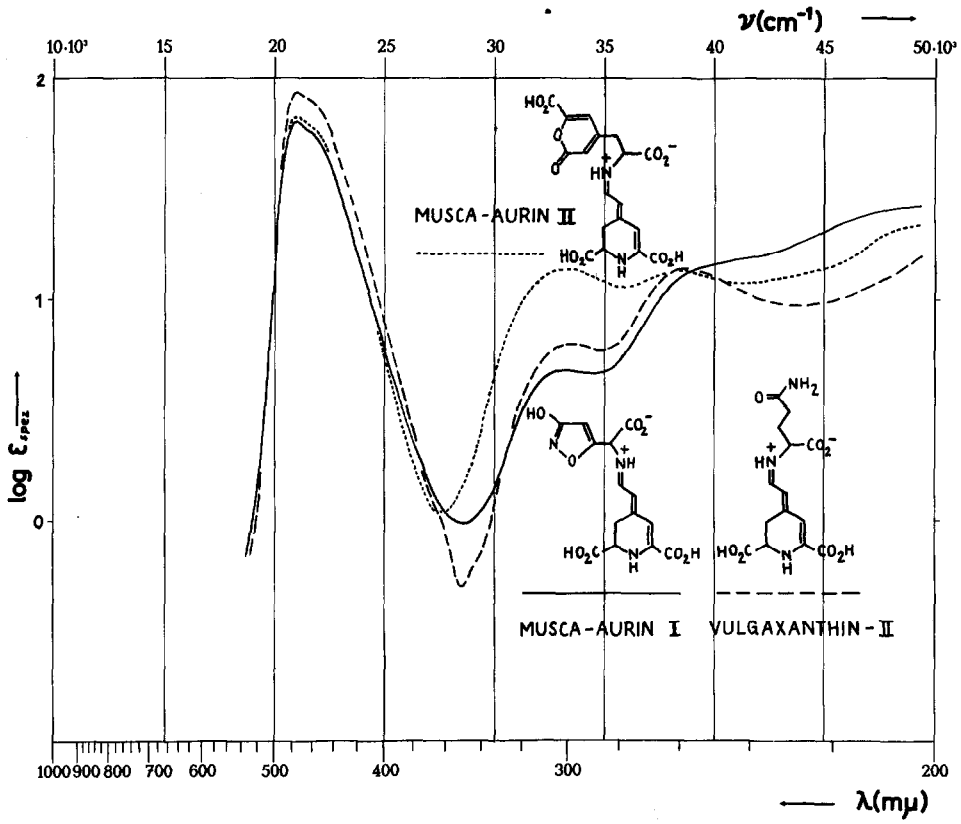

ν(cm⁻¹)
10·10³ 15 20 25 30 35 40 45 50·10³
log ε spez
MUSCA-AURIN II
MUSCA-AURIN I
VULGAXANTHIN-II
1000 900 800 700 600 500 400 300 200
λ(mμ)

Fig. 2.

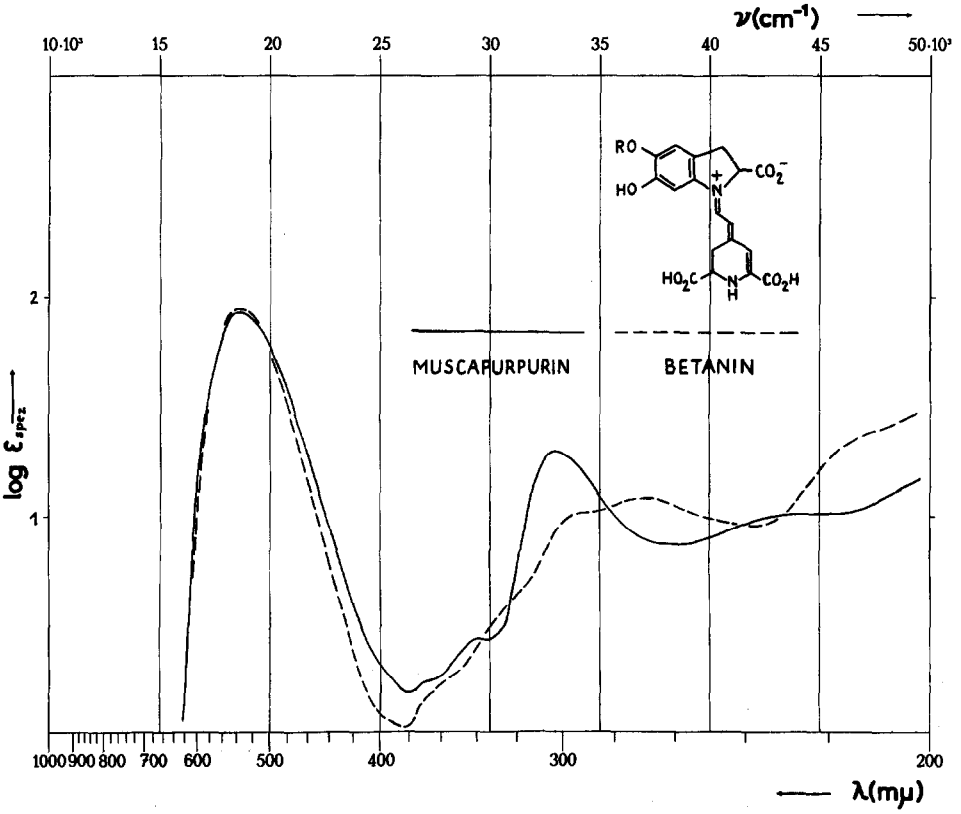
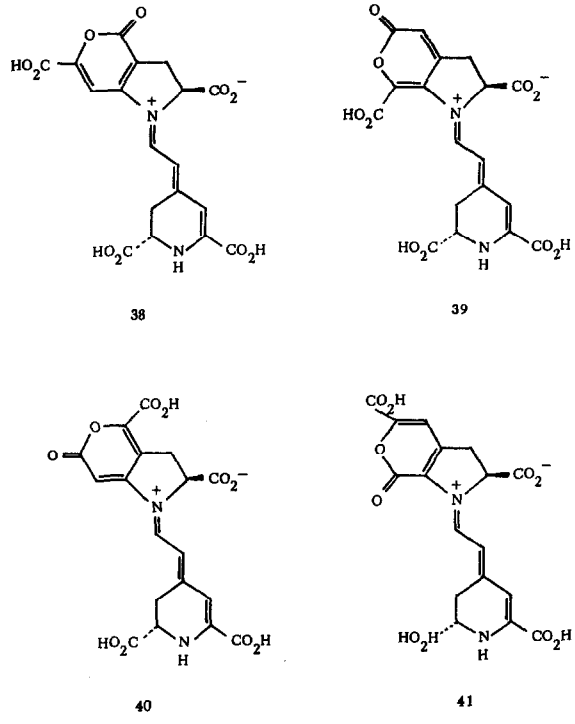
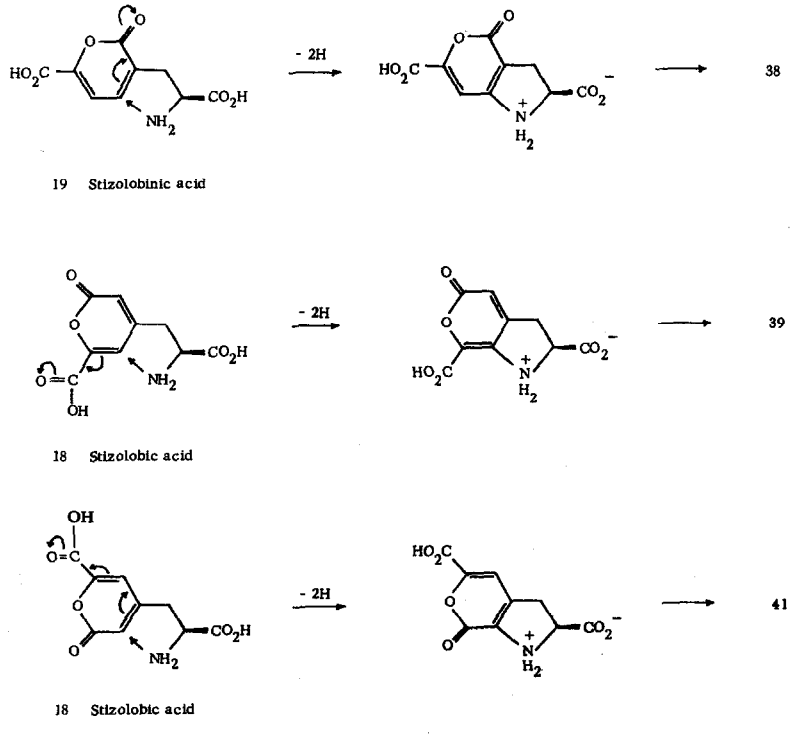

ν(cm⁻¹)
10·10³ 15 20 25 30 35 40 45 50·10³
log ε spez
MUSCAPURPURIN
BETANIN
1000 900 800 700 600 500 400 300 200
λ(mμ)

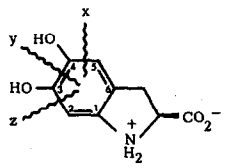
Fig. 3.



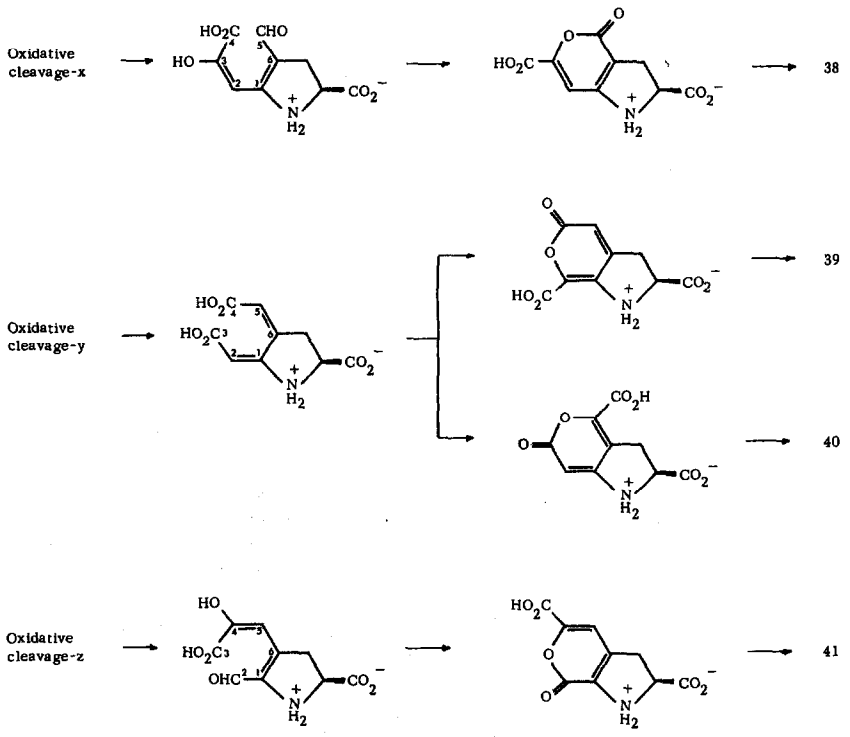
Scheme 4. Four possible structures for muscapurpurin.



Scheme 5. Biogenetic proposals leading to three possible structures for muscapurpurin.



42 Cyclodopa



Scheme 6. Biogenetic proposals leading to four possible structures for muscapurpurin.

be remarked that oxidative cleavage-x in Scheme 6 does correspond with cleavage-b in Scheme 1. The possibility that the cleavage-a in Scheme 1 and the corresponding cleavage-z in Scheme 6 *both operate in Amanita muscaria* makes the constitution (**41**) the favoured candidate for the structure of muscapurpurin at present. Attempts to solve this problem by synthesis are in progress.

Biosynthetic studies. Successful incorporation studies have shown that the biosynthesis of the betalains[28,29] and of stizolobic acid[32] can involve tyrosine and dopa as precurors. However, our attempts to develop fruiting bodies of *Amanita muscaria* by laboratory culture have not been successful, so we have had to rely upon the injection of ^{14}C-tyrosine into young toadstools growing in the forest. In one experiment,[43] enough colured material could be extracted to show that radioactivity was associated with the fraction (λ_{max} 475 nm). However, incorporation was low and further research on this topic will be carried out.

The phytochemical examination of Amanita *species and other fungi.* In the skins of *Amanita citrina, Amanita pantherina, Amanita rubescens, Amanita spissa,* and *Amanita fulva,* none of the pigments could be detected.[43] Only two orange pigments have been extracted from *Amanita flavoconia.*[44]

Amanita caesarea is a rare species found in Southern Germany and in Italy. It is not poisonous and is regarded as a delicacy. Its pigment profile[44] is interesting in that it contains all the pigments found in *Amanita muscaria* except muscaurin II (**28**). This is presumably also associated with the absence of ibotenic acid (**7**), which prevents the biosynthesis of muscaurin II (**28**).

The genus *Hygrocybe* has also provided an interesting result. Muscaflavin (**12a**) is present, but pigments (Schemes 3 and 4) derived from betalamic acid are absent. Presumably oxidative cleavage of bond-b (Scheme 1) is possible in this case, but cleavage of bond-a is not.

The sequel. During our investigation, many samples of *Amanita muscaria* have been examined. These were collected from forests in Sweden, Austria, Italy, Scotland, the United States of

America, and Germany (Westerwald, Spessart, Bayischer Wald, Schwarzwald, and the Harz Mountains). In all cases the pigment profiles were similar, although some variations in the relative concentrations of pigments were observed.[44]

Special attention was given to the mushrooms collected in the Harz Mountains because this was the region from which Kögl and Erxleben collected the samples from which they isolated muscarufin.[15] In our hands, no quinonoid materials could be detected. The only argument that I cannot dispute is that *Amanita muscaria* from the Harz Mountains has changed its pigment metabolism on its evolutionary path towards a higher plant. However, in my opinion, forty years is a rather short period for that to have occurred.

Acknowledgements—This work has been supported by the Deutsche Forschungsgemeinschaft and by the Fonds der Chemischen Industrie. Kind assistance in collecting starting material from different sources by many colleagues is acknowledged, and I thank Dr. W. S. Chilton for his kindness in donating authentic samples of stizolobic acid and stizolobinic acid.

I thank especially Prof. C. H. Eugster, Zürich, and Prof. W. Steglich, Bonn, for many discussions and ideal collaboration. In our Laboratory the most successful experimental work of Dr. H. Döpp is appreciated very much.

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