SCIENTIFIC AND TECHNICAL NOTES	SCITEC/6 February 1989

Chemistry and Reaction Mechanisms of Rapid Tests for Drugs of Abuse and Precursors Chemicals

Karl-Artur Kovar and Martina Laudszun

Pharmazeutisches Institut der Universität Tübingen
Auf der Morgenstelle 8, D-7400 Tübingen
Federal Republic of Germany

In 1987 the Division of Narcotic Drugs organized an expert group meeting in Vienna. This group recommended several rapid tests for the presumptive identification of controlled drugs and precursor chemicals in seizure (tab.1)[1,2]. For a most favourable application and improvement of the tests the knowledge of the chemical structures of the final products and of the reaction mechanisms is essential. Therefore this paper provides in a survey a simplified description of the formation of the coloring matters.

Table 1: Rapid Tests Selected for Field Use and for Laboratory Application

Drugs and Precursor Chemicals		Rapid Tests	
1	Opium	1.1	Marquis' Reagent: formaldehyde, glacial acetic acid/ conc. sulfuric acid (A)
		1.2	ferric sulfate (B)
2	Morphine, Codeine, Heroin	2.1	Marquis' Reagent: formaldehyde, glacial acetic acid/ conc. sulfuric acid (A)
		2.2	Mecke's Reagent: selenious acid, conc. sulfuric acid (C)
		2.3	nitric acid (D)
		2.4	ferric sulfate (B)
3	Methadone	3.1	Marquis' Reagent: formaldehyde, glacial acetic acid/ conc. sulfuric acid (A)
		3.2	nitric acid, sulfuric acid (E)

Drugs and Precursor Chemicals		Rapid Tests
4	Amphetamine/ · Methamphetamine and other amphetamine derivatives	4.1 Marquis' Reagent: formaldehyde, glacial acetic acid/ conc. sulfuric acid (*A*) 4.2 sulfuric acid (*F*) 4.3 Simon's Reagent: sodium nitroprusside, acetaldehyde/ sodium carbonate (*G*) 4.4 Simon's Reagent with acetone: sodium nitroprusside, acetone/ sodium carbonate (*H*) 4.5 Vitali—Morin's Reagent: fuming nitric acid/ acetone/ ethanolic potassium hydroxide (*N*) (recommended only for further studies)
5	Cannabis	5.1 Fast Blue B salt Reagent: Fast Blue B salt, sodium sulfate/ chloroform/ sodium hydroxide (*I*) 5.2 Duquenois-Levine's Reagent: vanillin, acetaldehyde/ conc. hydrochloric acid/ chloroform (*J*) 5.3 Ghamrawy's Reagent: p-dimethylamino benzaldehyde/ conc. sulfuric acid (recommended only for further studies)
6	Barbiturates	Dille-Koppanyi's Reagent: cobaltous acetate tetrahydrate, methanol, glacial acetic acid/ isopropylamine (*K*)

Drugs and Precursor Chemicals		Rapid Tests
7	Diazepam and other benzodiazepine derivatives	7.1 Zimmermann's Reagent: 1,3-dinitro-benzene/ potassium hydroxide (*L*)
		7.2 hydrochloric acid (*M*)
		7.3 Vitali-Morin's Reagent: conc. nitric acid/ acetone/ ethanolic potassium hydro-xide (*N*)
		7.4 trichloroacetic acid/ p-dimethylamino-cinnamic aldehyde (recommended only for further studies)
8	Lysergide (LSD)	Ehrlich's Reagent: p-dimethylamino benzaldehyde/ conc. orthophosphoric acid (*O*)
9	Cocaine	9.1 hydrochloric acid/ cobalt(II)-thio-cyanate (*P*)
		9.2 Modified Scott Reagent: cobalt(II)-thiocyanate, acetic acid, glycerine/ conc. hydrochloric acid/ chloroform (*R*)
		9.3 methanolic potassium hydroxide (*S*)
10	Methaqualone	hydrochloric acid/ cobalt(II)-thio-cyanate (*P*)
11	Phencyclidine (PCP)	11.1 hydrochloric acid/ cobalt(II)-thio-cyanate (*P*)
		11.2 Mecke's Reagent: selenious acid, conc. sulfuric acid (*C*)

Drugs and Precursor Chemicals		Rapid Tests
12 Anthranilic acid·	12.1	Ehrlich's Reagent: p-dimethylamino benzaldehyde, conc. orthophosphoric acid (O)
	12.2	Simon's Reagent: sodium nitroprusside acetaldehyde/ sodium carbonate (G)
13 Benzyl-methyl ketone (P2P)	13.1	Marquis' Reagent: formaldehyde, glacial acetic acid/ conc. sulfuric acid (A)
	13.2	hydrochloric acid/ cobalt(II)-thio-cyanate (P)
	13.3	Zimmermann's Reagent: 1.3-dinitro-benzene/ potassium hydroxide (L)
14 Ergotamine		Ehrlich's Reagent: p-dimethylamino
15 Ergometrine		benzaldehyde, conc. orthophosphoric
16 Lysergic acid		acid (O)
17 Ephedrine/ Norephedrine		Chen-Kao's Reagent: acetic acid/ copper sulfate/ sodium hydroxide (T)
18 Piperidine		Simon's Reagent: acetaldehyde, sodium nitroprusside (G1)

1 Opium

1.1 The color reaction with Marquis' R results in a purple to violet coloring. According to Auterhoff and Braun[3] two molecules morphine (1) and two molecules formaldehyde condense in presence of conc. sulfuric acid to the dimeric product 2, which is protonated to the oxonium-carbenium salt 3a/b.
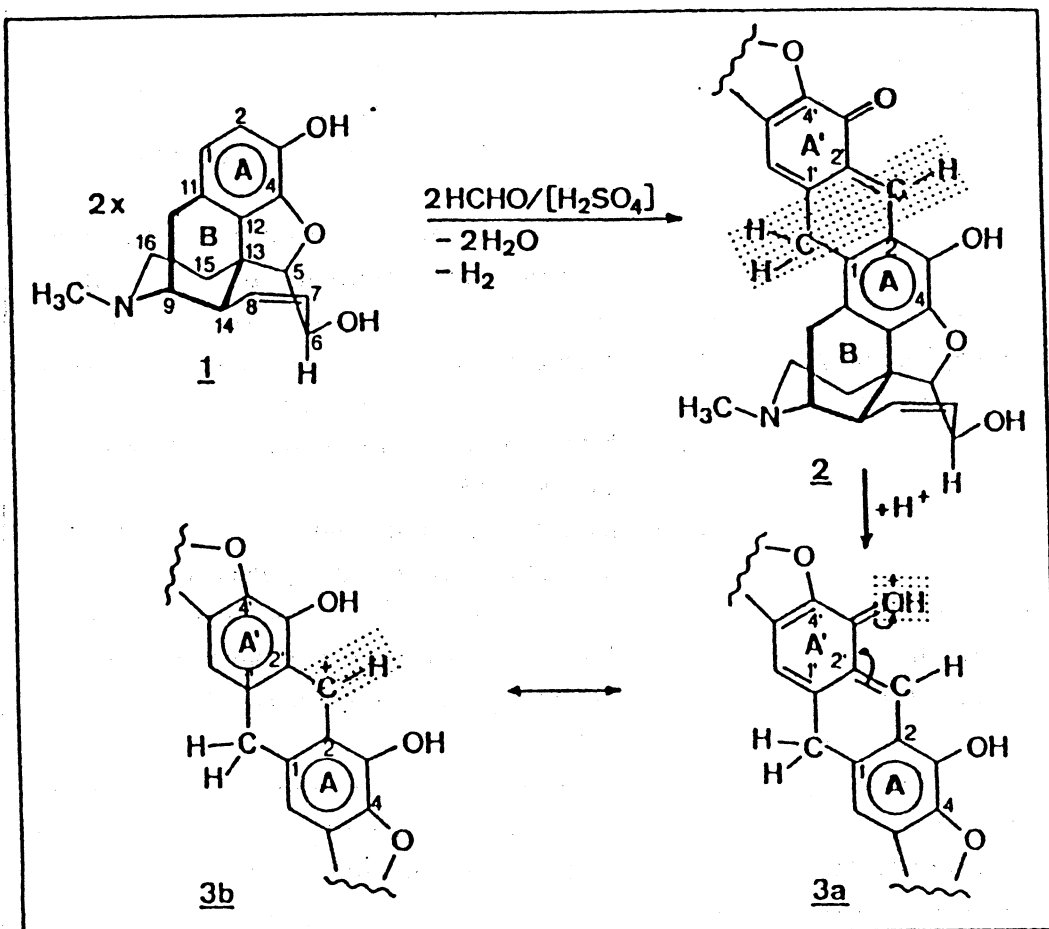
Fig. 1: Marquis reaction of morphine

1.2 **Ferric Sulfate Test** (cf. 2.4)

2 Morphine, Codeine, Heroin

2.1 **Marquis Test** (cf. 1.1)

2.2 The coloring matter (blue to green) produced by **Mecke's** R may be explained analogous to the Husemann-Reaction[4]. In this case morphine (1) is rearranged to apomorphine (4), which in presence of selenious acid is oxidized to the o-quinone of apomorphine (5).
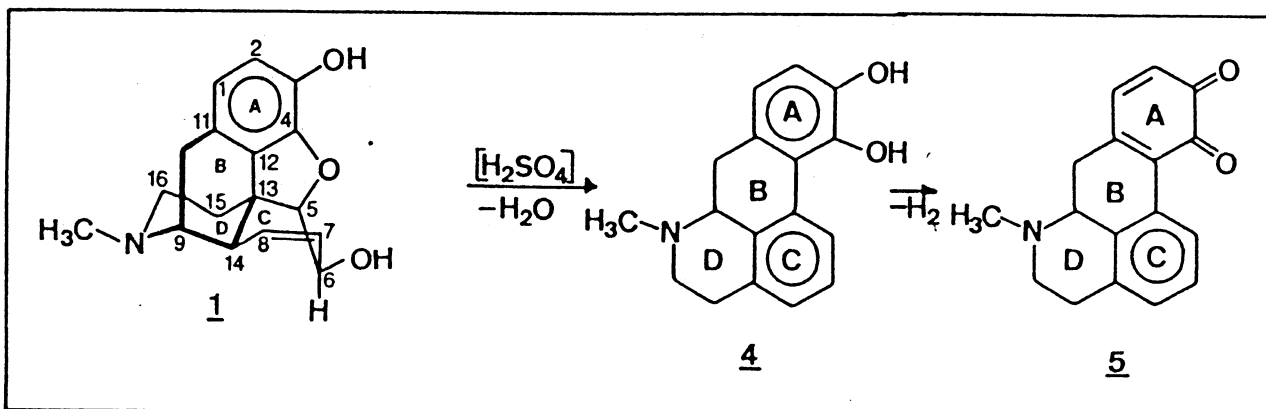
Fig. 2: Proposed mechanism of the color reaction of morphine with
Mecke's R

2.3 The reaction with <u>conc. nitric acid</u> makes it possible to differentiate between morphine (orange red color) and its O-substituted derivatives at C-3, e.g. codeine (orange color) and heroin (yellow color). Both substituted and non-substituted derivatives are nitrated in position 2 (<u>6a/b</u>). The nitroproduct of morphine forms a hydrogen bond between the nitro group and the hydroxyl group (<u>6a</u>). This is impossible in the case of O-substituted derivatives (<u>6b</u>)[5)14)].

<u>6a</u>   <u>6b</u>
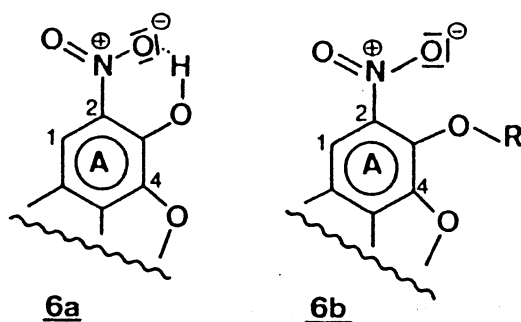
2.4 <u>Ferric sulfate</u> is used for the determination of the meconic acid as a constituent of opium. The enolic structure leads to chelate formation <u>7</u> which is responsible for the purple color[6)7)].

<u>7</u>
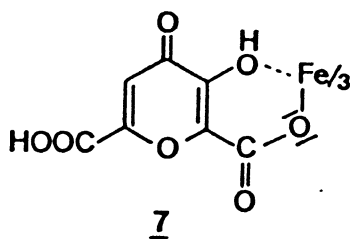
3 Methadone

3.1 Marquis' R is used for a qualitative determination of aromatic substances, e.g. methadone (8). The carbonium ion 9, which is formed from formaldehyde, reacts with the aromatic structure of the compound. Under the influence of sulfuric acid the carbenium ion 11 is produced and is stabilized by reacting with a second molecule of the aromatic component. Traces of heavy metal in sulfuric acid, especially iron, are responsible for the oxidation of 12 to 13. Due to the acidic medium 13 is converted to the pink-colored carbenium ion 14[8].
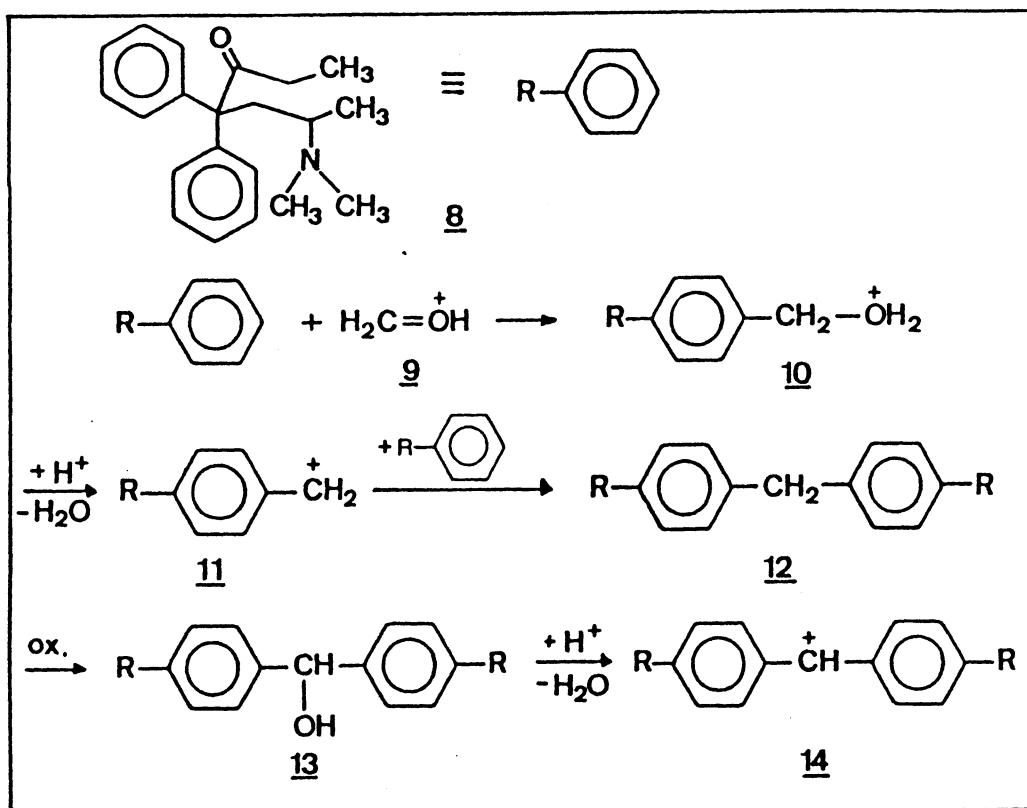
Fig. 3: Marquis reaction of aromatic compounds

3.2 The product of the reaction of methadone with conc. nitric acid and conc. sulfuric acid has not yet been clarified. The reaction results in the formation of an orange color[9].

4 Amphetamine/Methamphetamine and other amphetamine derivatives

4.1 Analogous to methadone (3.1), amphetamine (15) forms an orange-, methamphetamine a yellowish green-colored product with Marquis' R. The carbenium ion 16 is held to be responsible for the color produced[8]. Some other ampethamine derivatives also yield yellow, yellowish green and other colors.
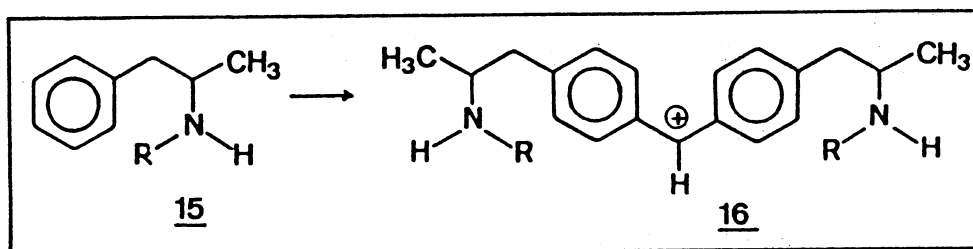

Fig. 4: Marquis reaction of amphetamine (R= H) and methamphetamine (R= CH₃)

4.2 According to Neuninger[10] it is possible to differentiate between amphetamine derivatives which are substituted at the aromatic part of the structure and those which are not substituted e.g. amphetamine and methamphetamine. With conc. sulfuric acid colored products are only formed by substituted derivatives. The structures of these products as well as the mechanism of the reaction have not yet been clarified.

4.3 Secondary amines, e. g. methamphetamine, can be identified with Simon's R by forming a blue Simon-Awe complex. The amine and acetaldehyde produce the enamine 17, which subsequently reacts with sodium nitroprusside to 18. Finally, the immonium salt 18 is hydrolized to the Simon-Awe complex 19[11].
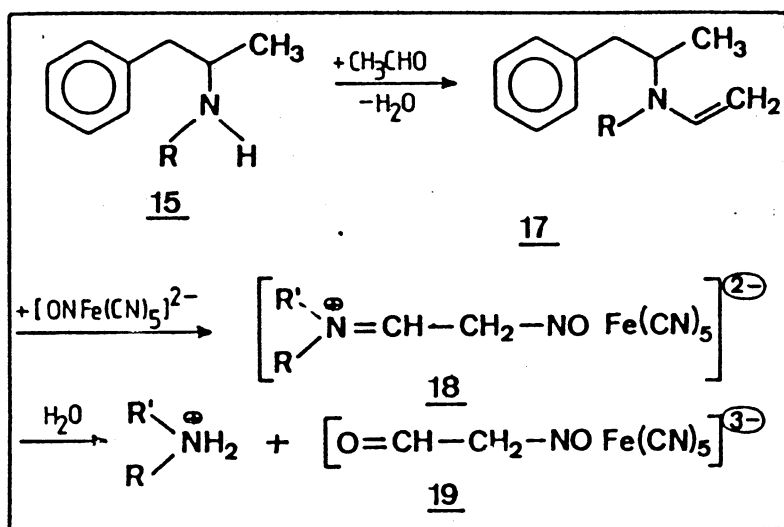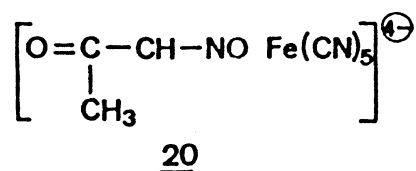
Fig. 5: Simon-Awe reaction of secondary amines

4.4 If acetaldehyde is replaced by <u>acetone</u>, the <u>Simon's R</u> works selectively with primary amines. Analogous to the Legal Reaction a purple-colored product <u>20</u> is obtained[12].

$$\left[O=\underset{\underset{CH_3}{|}}{C}-CH-NO \ \ Fe(CN)_5 \right]^{4\ominus}$$

<u>20</u>

4.5 In the course of the <u>Vitali-Morin reaction</u> green-, purple- or blue-colored salts of nitro products (<u>21</u>) are formed by those amphetamine derivatives which are not substituted at the aromatic structure. The quantity of the added base determines the color. Methoxy- and methylenedioxy-derivatives produce either a green color, or the result of this reaction is negative, i.e. a yellowish color with a brown precipitate is obtained[13].

<u>21</u>
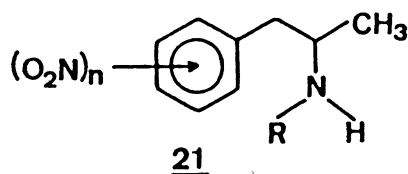
5   Cannabis products

5.1   The chloroform extract of cannabis products (hashish, marihuana) reacts with _fast blue salt B_ in a basic milieu by forming the coupled product 22 (purple-red), which is soluble in the organic layer. Using _fast blue salt BB_, 23 is formed[5)14)].
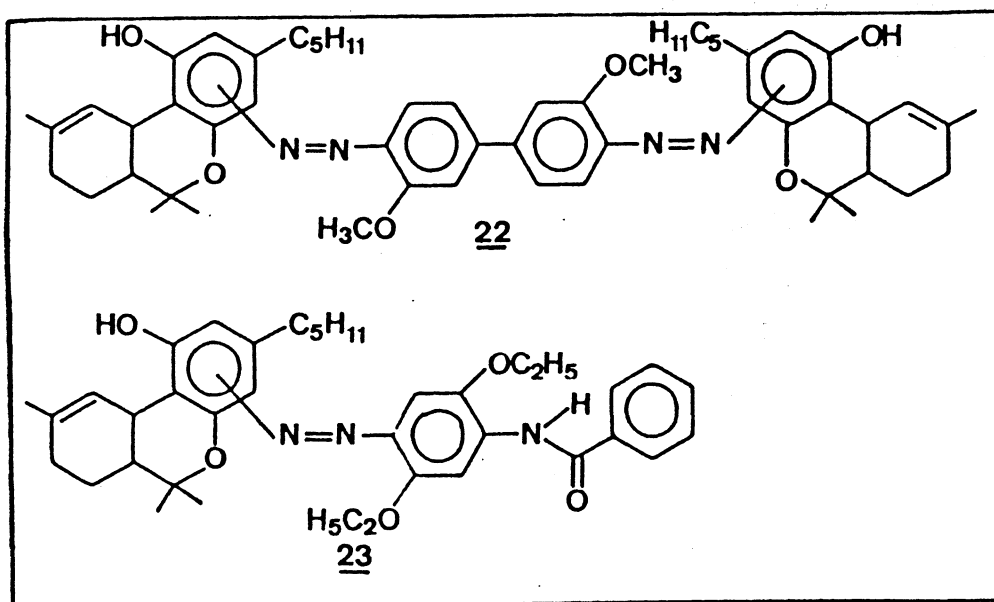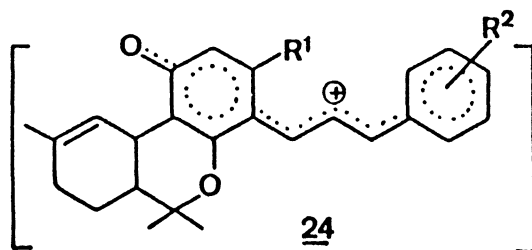

Fig. 6: Reaction of cannabis with fast blue salts

5.2   The _Duquenois-Levine_ Test describes the determination of cannabis resin with vanillin and acetaldehyde in a hydrochloric medium by forming the violet-colored product 24, which can be extracted with chloroform[15)]. The mechanism of this reaction will be subject of work by Krieger[16)].

5.3   The rapid analysis of cannabis products according to Ghamrawy results in the formation of a red color, which turns into blue when diluting the sample with water. Cannabidiol, THC and the acids of these

compounds are responsible for this reaction, but not cannabinol or cannabinolic acid[17]. With conc. sulfuric acid the tertiary carbenium ion **25** and later the cyclohexenyl carbenium ion **26a** is formed by 1,3 displacement of a hydride ion. **26a** is in equilibrium with **26b** (diene). **26b** reacts with p-dimethylamino benzaldehyde in the presence of conc. H_2SO_4 to the polymethin carbenium ion **27** (Kriewitz-Prins Reaction). The change of color effected by the dilution with water is due to the deprotonation of the dimethylamino group to **28**[17].
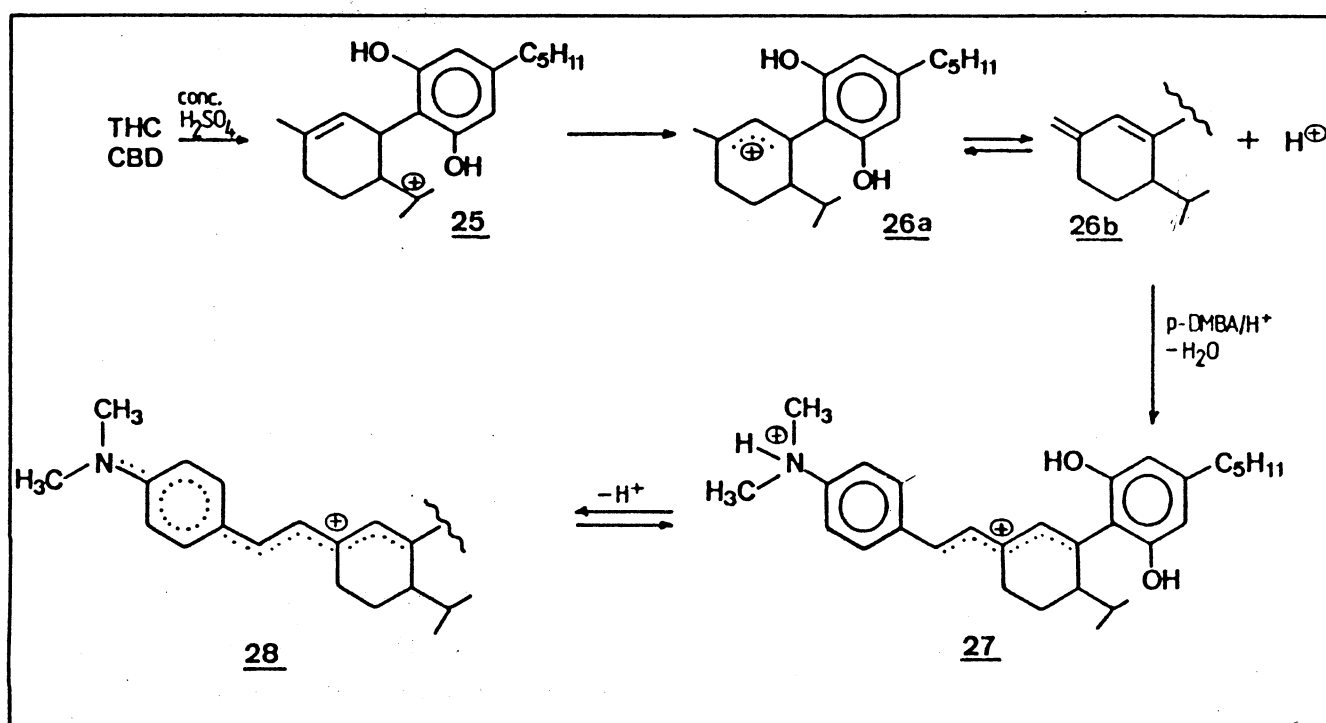

Fig. 7: Ghamrawy reaction of cannabis constituents

6   Barbiturates

N-non-substituted barbiturates can be detected with Dille-Koppanyi's R. Isopropylamine is responsible for the deprotonation of the barbiturate molecule. The purple color is caused by a complex formation of two molecules barbiturate and one cobalt cation. Two molecules of isopropylamine act as stabilizers of this complex **29**[18][19].
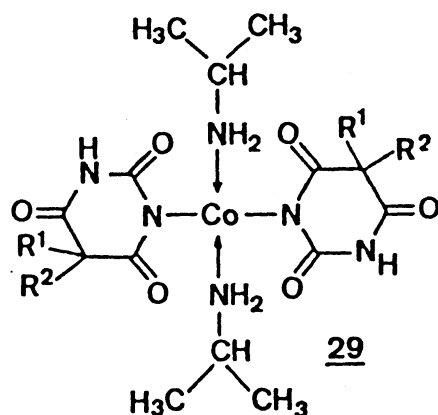
7   Diazepam and other benzodiazepine derivatives

7.1 The essential condition for the color reaction of benzodiazepines with Zimmermann's R is an activated methylene group at C-3, i.e. C-2 must have a carbonyl group and N-1 an alkyl group. A positive result of the reaction is also achieved with triazolo-benzodiazepines. In alkaline medium with m-dinitrobenzene a reddish-purple Meisenheimer complex (31) is formed via the carbanion 30[20)-22)].
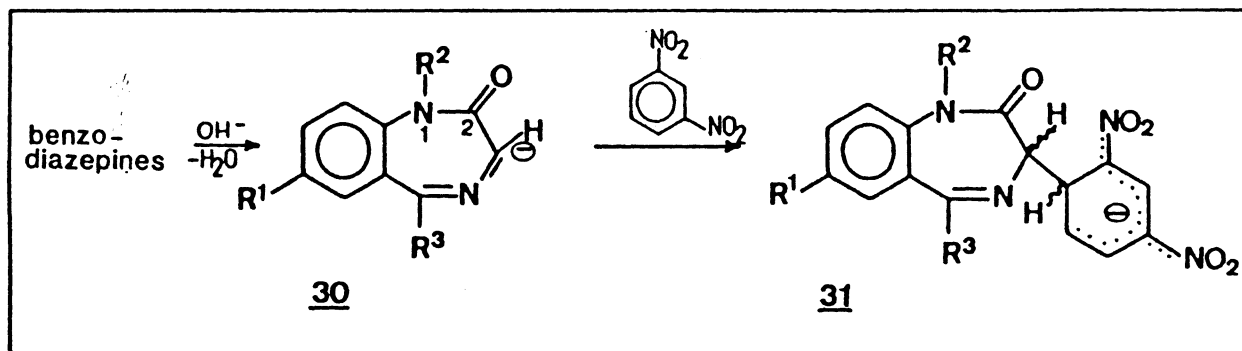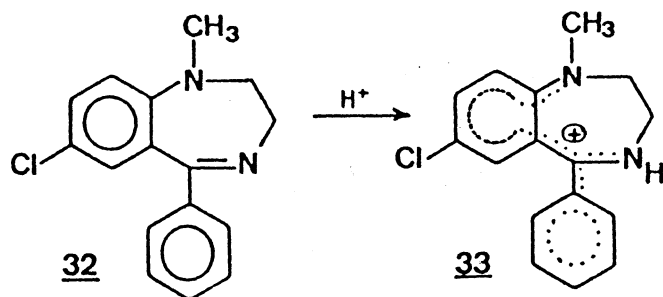

Fig. 8: Zimmermann reaction of benzodiazepines

7.2 With conc. hydrochlorid acid most of the benzodiazepines develop a yellowish coloring, which is probably due to a benzophenone structure. In the case of medazepam (32), however, the red phenylogous amidinium cation 33 is formed by heating with hydrochlorid acid[21)].

32 → **33**

7.3 The prescribed <u>Vitali-Morin reaction</u> does not provide any specific results for benzodiazepine derivatives. After heating the substances to dryness with con. nitric acid a yellow-orange color is produced in acetone by alkali. The color may be caused by the formation of nitrated benzophenone derivatives.

7.4 Both the mechanism and the structure of the red product resulting from the reaction with <u>trichloroacetic acid</u> and <u>p-dimethylamino cinnamic aldehyde</u> have not yet been clarified[23].

8 Lysergide (LSD)

Indol derivatives like LSD or lysergic acid (cf. 16), ergotamine (cf. 14) and ergometrine (cf. 15), which do not have any substitution in position 2 (**34**) are attacked by Ehrlich's R at C-2. The coloring matter produced is determined by the concentration of the acid, the solvents and other conditions of the reaction. After the condensation of one molecule LSD with one molecule p-dimethylamino benzaldehyde the carbinole **35** is formed. After protonation H_2O is eliminated to form the carbenium ion **36**, which reacts to **37** by the addition of a second molecule LSD. **37** is oxidized to the blue-colored cyanine **38**[24].
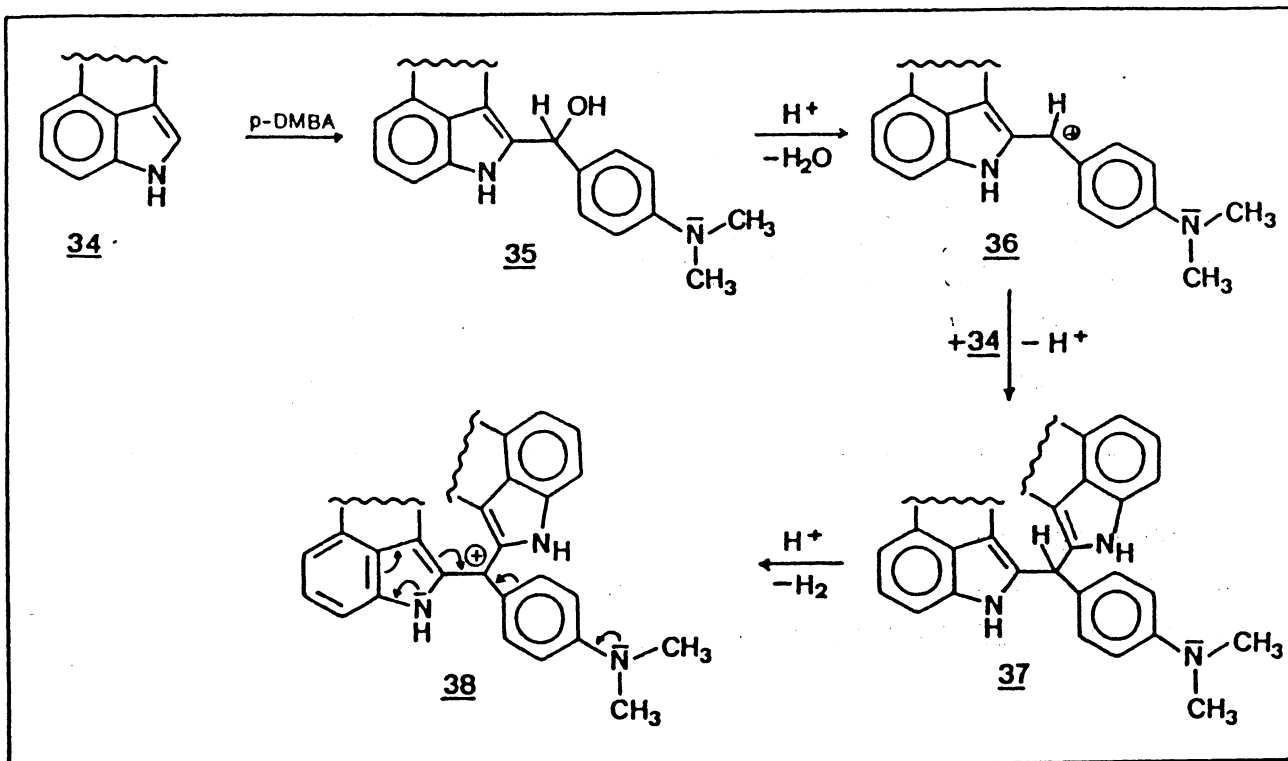
Fig. 9: Reaction of indole derivates with Ehrlich's R

9   Cocaine

9.1   The Young Test describes the reaction of cocaine with cobalt(II)-thiocyanate in acidic milieu. The exact structure of the blue-colored cocaine-cobalt-thiocyanate complex is not known yet[14].

9.2 The Scott Test has been developed and modified to differentiate between cocaine in base or salt form and other compounds (e.g. procaine, lidocaine, tetracaine) which give false positive results by using the cobalt(II)-thiocyanate reagent (cf. 9.1). In this modified Scott Test only the cobalt(II)-thiocyanate complex of cocaine is extracted with chloroform. The cobalt(II)-thiocyanate complex of PCP can also be extracted by chloroform. But in contrast to cocaine it does not produce a pink intermediate color when hydrochloric acid is added.

9.3   Cocaine (39) is hydrolized to 40 by treatment with methanolic potassium hydroxide (or potassium methylate) and can be recognized by the characteristic smell of the methyl benzoate   (41)[29].

$$\underset{\textbf{40}}{} \xrightarrow[\text{[NaOH + CH}_3\text{OH]}]{\text{NaOCH}_3} \underset{\textbf{41}}{}$$
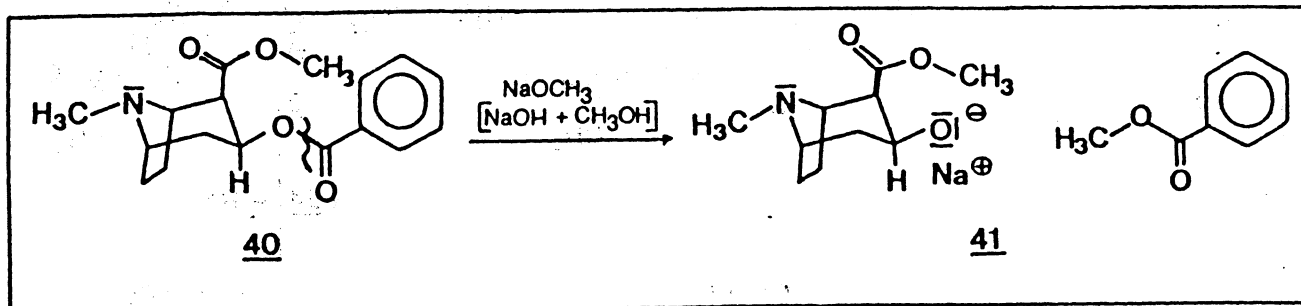
10   Methaqualone

Analogous to cocaine, methaqualone produces a blue-colored unidentified product with cobalt(II)-thiocyanate in hydrochloric acid medium.

11   Phencyclidine (PCP)

11.1 Phencyclidine and cobalt(II)-thiocyanate react in hydrochloric acid by forming a similar blue-colored unidentified complex as cocaine (cf. 9.1) and methaqualone (cf. 10).
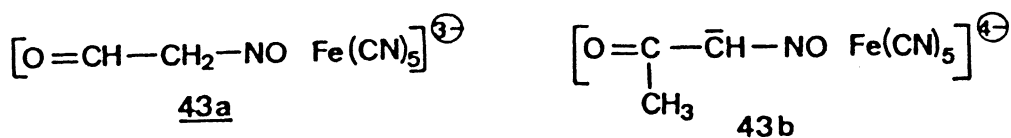
11.2 Moreover it is possible to identify phencyclidine by adding Mecke's R, but the reaction mechanism has not yet been clarified. The color of the sample turns into pink.

12   Anthranilic acid

12.1 The primary amino group of the anthranilic acid molecule forms a red-colored azomethine 42 with Ehrlich's R[25].
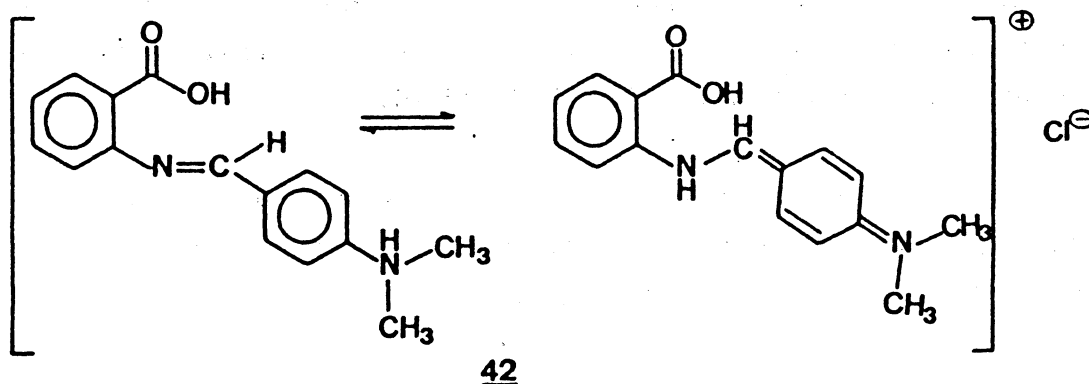
12.2 As has been described above (4.3 and 4.4), Simon's R yields a positive result only with secondary amines (e.g. N-substituted anthranilic acid derivatives), whereas Simon's R with acetone is appropriate for the identification of primary amines (e.g. non-substituted anthranilic acid). 43a shows the product of Simon's Reaction with acetaldehyde, 43b that with acetone[11][12].

$$\left[O=CH-CH_2-NO \ \ Fe(CN)_5 \right]^{3\ominus}$$
43a

$$\left[O=\underset{\underset{CH_3}{|}}{C}-\overline{C}H-NO \ \ Fe(CN)_5 \right]^{4\ominus}$$
43b

13 Benzyl-methyl ketone (P2P)

13.1 Benzyl-methyl ketone can be identified with Marquis' Reagent (cf. 3.1) by forming an orange-yellow color. First a few intermediates are produced, which are then transformed into a carbinol and finally to the orange-yellow-colored product 14.[8]

13.2 The reaction with cobalt(II)-thiocyanate and hydrochloric acid results in a blue product with unknown structure.

13.3 Another possibility for the determination of benzyl-methyl ketone is the condensation with Zimmermann's R. In alkaline medium dinitrobenzene reacts with the methyl group, which is activated by the keto group. The supposed reaction mechanism is shown below[26][27]. As a first step the Meisenheimer complex 44 is formed. 44 may be oxidized to the Zimmermann compound 45 by an excessive amount of dinitrobenzene.
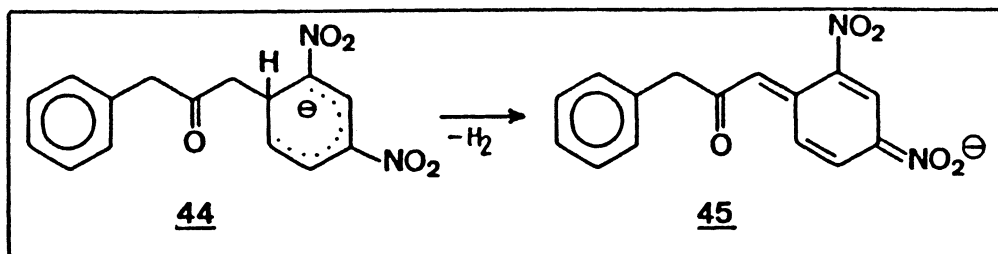

Fig. 10: Zimmermann reaction of benzyl-methyl ketone *(P2P)*

14 - 16 Ergotamine, Ergometrine, Lysergic acid

These substances condensate with Ehrlich's R thus forming violet cyanine coloring matters like **38** (cf. 8).

17 Ephedrine/ Norephedrine

The Chen-Kao Reaction is selective for phenylalkylamines with vicinal amino- and hydroxyl-groups (**46**). The result is a violet-colored copper chelate complex **47**[28], which can be extracted with n-butanol.
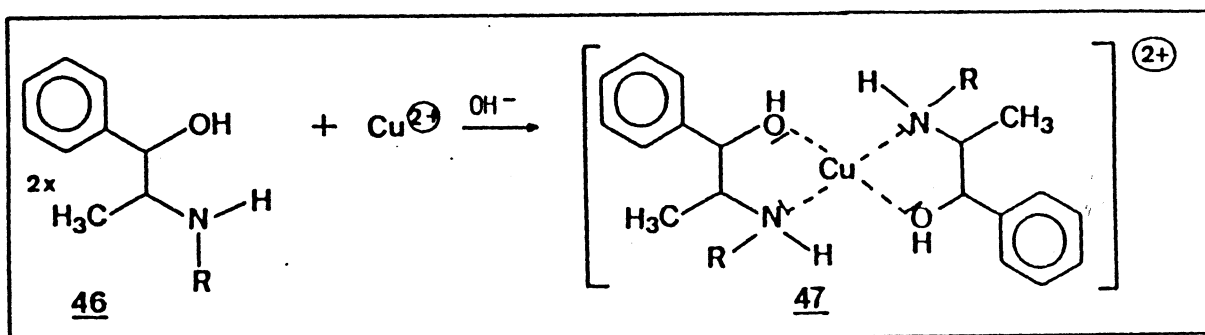

Fig. 11: Chen-Kao reaction of phenylalkylamine.

18 Piperidine

According to Wiegrebe and Vilbig[11][12], piperidine can be detected by Simon's R because of its secondary amino group (cf. 4.3). A blue colored Simon-Awe complex **50** is formed via **48** and **49**.
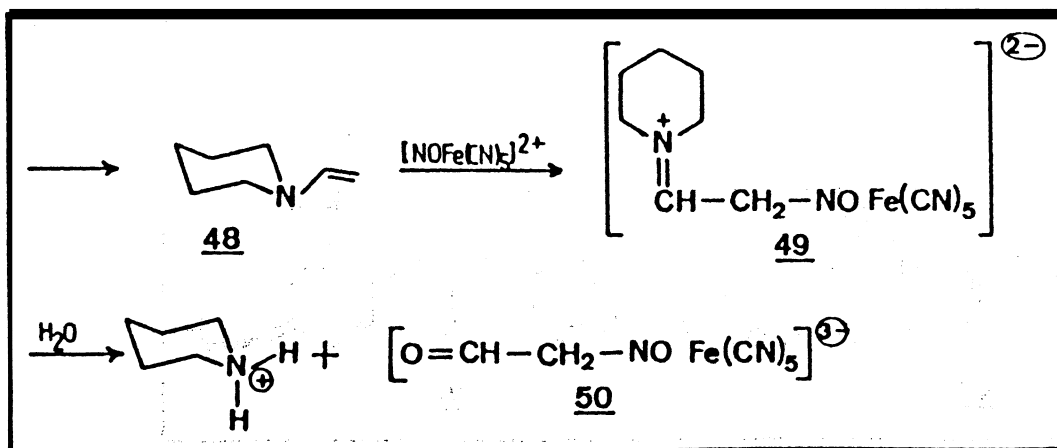

Fig. 12: Reaction of piperidine with Simon's R.

References

1) Report of the Expert Group on Rapid Testing Methods of Drugs of Abuse (1988) E/CN 7/CRP 3, United Nations, Vienna

2) Rapid Testing Methods of Drugs of Abuse, Manual for Use by National Law Enforcement and Narcotics Laboratory Personnel (1988) ST/NAR/13, United Nations, New York

3) Auterhoff H, Braun D (1973) Arch Pharm (Weinheim) *306*: 866

4) Rehse K (1969) Arch Pharm (Weinheim) *302*: 487

5) Ditzel P, Kovar K-A (1983) Rausch- und Suchtmittel, Dtsch Apotheker Verlag, Stuttgart

6) Hartke K, Mutschler E (eds) (1987) DAB 9-Kommentar, Wissenschaftl Verlagsges, Stuttgart: 2603

7) Roth HJ, Eger K, Troschütz R (1985) Pharmazeutische Chemie II Arzneistoffanalyse 2 Aufl, Georg Thieme, Stuttgart, New York: 517

8) Brieskorn CH, Reiners W, Kiderlen H (1965) Arch Pharm (Weinheim) *298*: 505

9) Demonceau J (1952) J Pharm Belg *7*: 36

10) Neuninger H (1987) Sci Pharm *55*: 1

11) Wiegrebe W, Vilbig M (1981) Z Naturforsch *36b*: 1297

12) Wiegrebe W, Vilbig M (1982) Z Naturforsch *37b*: 490

13) Grausam U, forthcoming PhD thesis

14) Kovar K-A, Noy M, Pieper R (1982) Dtsch Apoth Ztg *122*: 3

15) Kovar K-A, Keck M, Krieger Th (1988) Sci Pharm *56*: 29 and Arch Pharm (Weinheim) *321*: 249

16) Krieger Th, forthcoming PhD thesis

17) Kovar K-A, Keilwagen S (1984) Arch Pharm (Weinheim) *317*: 724

18) Koppanyi T, Dille JM, Murphy WS, Krop S (1934) Pharm Assoc *23*: 1074

19) Hartke K, Mutschler E (eds) (1987) DAB 9-Kommentar, Wissenschaftl Verlagsges, Stuttgart: 97

20) Kovar K-A, Biegert B (1976) Arch Pharm (Weinheim) *309*: 522

21) Kovar K-A, Linden D (1983) Pharm Acta Helv *58*: 66

22) Kovar K-A, Kaiser C (1986) Pharm Acta Helv *61*: 42

23) Laudszun M, forthcoming PhD thesis

24) Pindur U (1982) Pharm Unserer Zeit *11*: 74 and private communication (1989)

25) Kakác B, Vejdelek ZJ (eds) (1974) Handbuch der photometrischen Analyse organischer Verbindungen Bd 2, Verlag Chemie, Weinheim: 499

26) Kakác B, Vejdelek ZJ (eds) (1974) Handbuch der photometrischen Analyse organischer Verbindungen Bd 1, Verlag Chemie, Weinheim: *275*

27) Kovar K-A (1972) Pharm Unserer Zeit *1*: 17

28) Hartke K, Mutschler E (eds) (1987) DAB 9-Kommentar, Wissenschaftl Verlagsges, Stuttgart: 1550

29) Grant FW, Martin WC, Quackenbush RW (1975) Bull Narc *27*: 33