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Note

Group-specific detection reagents for some 5- and/or 8-substituted 2-amino-3-hydroxytetralin derivatives

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2-Aminotetralins are pharmacologically active substances that occur as structural fragments of a number of alkaloids (morphine, etc.) and are structurally related to sympathomimetic amines.

Dantchev, Christova and co-workers have studied 2-amino-3,5,8-trihydroxytetralin derivatives that exhibit vasoconstrictory, vasodilatatory, antiarrhythmic and β-blocking effects[1–3]. Of these a pressor substance, *trans*-2-hydroxyethylamino-3-hydroxy-5,8-dimethoxy-1,2,3,4-tetrahydronaphthalene (provisionally called tetraminol; compound **2** in Table I) seems to be therapeutically promising.

As a part of pharmacological study of a drug, it is imperative to establish its fate in the body. Of the metabolic conversions of **2**, O-demethylation, oxidation of the alcohol group and chemical reactions involving the nitrogen atom may by considered. Evidence for O-demethylation was obtained in preliminary experiments with liver microsomes.

Methods of detection and chromatographic separation must be available for the characterization of the metabolites of compound **2**. This substance fluoresces with an excitation maximum at 284 nm and an emission maximum at 330 nm[4]. These properties cannot be utilized for thin-layer chromatographic (TLC) detection.

One would expect that periodate (or periodic acid, HIO_4) would attack the hydroquinones and their monomethyl ethers[5,6] and the primary or secondary 2-amino-3-hydroxy moieties[7,8] of these compounds, at least under certain conditions. Periodate was therefore tested as a component of most of the reagents examined. Procedures resulting in reactions selective for some of the functional groups of these compounds are reported here.

EXPERIMENTAL

Materials

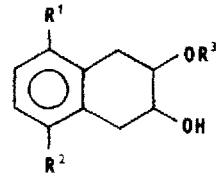
Commercial products (pure or analytical-reagent grade) were used unless stated otherwise.

Silufol silica gel layers (Kavalier) or layers prepared in the laboratory from Kieselgel 60H (Merck), Alufol alumina layers (Kavalier) and Lucefol cellulose layers (Kavalier) were used.

0021-9673/84/$03.00

TABLE I

1,2,3,4-TETRAHYDRO- AND 1,4-DIHYDRONAPHTHALENE DERIVATIVES TESTED



Except for **1** *cis*, all compounds are 2,3-*E* (*trans*) isomers. In cases where the compounds cannot be easily characterized by means of the R^1, R^2 and R^3 symbols, full formulae are given.

R^3 (given in the table)

*$R^1 = R^2 = OCH_3$**

1 *cis* }
1 *trans* } $-NH_2$

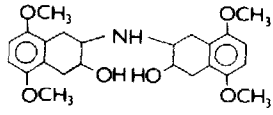
2 $-NHCH_2CH_2OH$

3 $-NHCH_2CH_2CH_2OH$

4 $-NH-C_6H_5$

5 $-NHCH_2COOH$

6

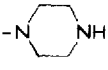


7 $-N(CH_3)_2$

8 $-N(CH_3)CH_2CH_2OH$

9

10



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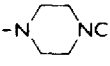

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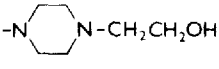

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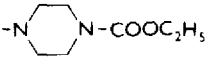

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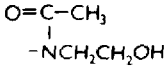

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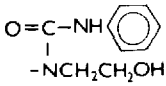

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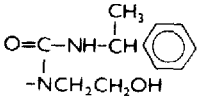

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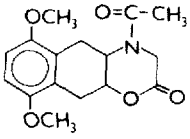

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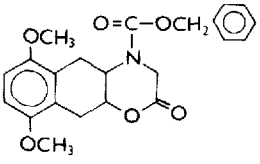

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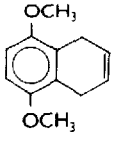

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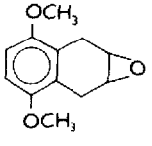
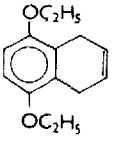


22 $-Cl$

23 $-OH$

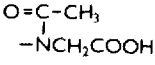
24



25 $-NHCH_2CH_2OH$

26 $-NHCH_2COOH$

27



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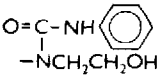

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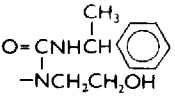

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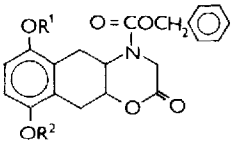

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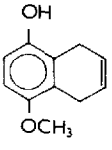
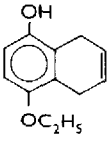


32 $-OH$

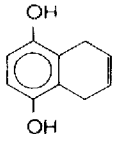
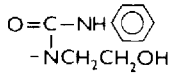
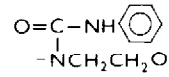
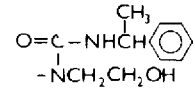
33



$R^1 = OH$ $R^2 = OCH_3$ or,** $R^1 = OCH_3$ $R^2 = OH$*

* Except for the ethoxy compounds **24** and **33**.

** Mixtures of 5-hydroxy-8-alkoxy and 8-hydroxy-5-alkoxy isomers of the compounds included in these columns were tested. In cases where the isomers were chromatographically resolved, all the reactions under test gave identical results for the respective pairs. Accordingly, the isomers will not be differentiated in either the table or the text.

$R^1 = R^2 = OH$	$R^1 = H \quad R^2 = OCH_3$ *or*** $R^1 = OCH_3 \quad R^2 = H$	$R^1 = OH \quad R^2 = H$ *or*** $R^1 = H \quad R^2 = OH$
34 $-NHCH_2CH_2OH$		**37** $-NHCH_2CH_2OH$
35 	**36** 	**38** 
		39 

The substances investigated are shown in Table I. Compounds **1–7, 9–14, 20–22, 34** and **35** were kindly donated by Dr. K. Christova of the Department of Organic Chemistry, Faculty of Pharmacy, Medical Academy, Sofia, Bulgaria. The remaining compounds were prepared in our laboratory[9].

Substances without substitution in the aromatic ring are not included in Table I (see fourth paragraph of Discussion).

Reagents producing coloured spots

D1. 1 g of $HIO_4 \cdot 2H_2O$, 10 ml of ethanol and 10 ml of diethyl ether. After spraying, heated at 50°C (for time, see Results).

D2. As for D1, but heated at 50°C until the maximum intensity of the yellow or ochre colour is reached. Followed by spraying with:

(a) 25% aqueous ammonia;
(b) 16% 1-butylamine in chloroform;
(c) 16% 1-butylamine in acetone;
(d) 15% morpholine in chloroform;
(e) 15% morpholine in acetone;
(f) triethylamine;

D3 a–f. Spraying with amines as in D2a–f, without the preceding reactions with HIO_4 and without heating.

Reagents producing fluorescent spots (excitation at 365 nm)

D4. 0.2 g of KIO_4, 0.5 g of NaOH, water to make 50 ml.

D5(A). 1 ml of paraffin oil, 1 ml of 1-butylamine, made up to 10 ml with diethyl ether.

D5(B). 25 mg of $KMnO_4$ made up to 100 ml with water.

After spraying with D5A, the chromatogram is left until dry and subsequently lightly sprayed with D5B. Fluorescence may be observed immediately.

RESULTS

With Lucefol reagents D1 and D2 were less sensitive with poorly defined colours. Reactions with reagents D3–D5 may be performed even on cellulose. With D1 there were only slight differences in hue between the silica and alumina layers.

Detection limits will be reported for Silufol. When expressed per unit surface area, the sensitivities were almost identical for the spot tests and the chromatographic spots.

D1. All the compounds yield yellow or ochre colours with maximum intensity (for different compounds) between 30 sec and 3 min. The detection limit is 2 $\mu g/cm^2$.

D2. The results are summarized in Table II. Whereas butylamine in chloroform (D2b) (or in methanol) yields a different colour from butylamine in acetone (D2c), morpholine in chloroform (D2d) (or in methanol) gives colours identical with those in acetone (D2e). The brownish purple colour of the compounds of the latter group (B) with D2d and D2e (morpholine) is relatively stable, whereas with D2b it turns brown within about 15 min.

D3a–f. Colour reactions occur only with the *p*-diphenols (**34** and **35**), namely pink (within 15 min) for D3a, D3d and D3e, brownish purple turning to orange

TABLE II

REACTIONS WITH D2a f (COLOUR) AND D4 (FLUORESCENCE, EXCITATION AT 365 nm)

Type	*Substances*	*Compounds (see Table I)*	*D2a, D2f*	*D2b, D2d, D2e*	*D2c*	*D4*
A	Primary and secondary amines and 2,3-diols	**1** (*cis*), **1** (*trans*); **2-6, 37; 23**	Blue-violet ↓ 15 min	Olive green Detection limit 2 $\mu g/cm^2$		Yellowish fluorescence Detection limit 0.1 $\mu g/cm^2$
		25, 26, 34; 32	Ochre			No fluorescence
B	Tertiary and acylated secondary amines and some other compounds	**7-19, 27-30, 36, 38, 39; 20-22, 24; 31, 33, 35**	Brown	Brownish purple ↓ 15 min (D2b) Brown* Detection limit** 0.6 $\mu g/cm^2$	Blue ↓ 15 sec -3 min Olive green Detection limit** 0.35 $\mu g/cm^2$	No fluorescence

* Orange with **20, 24, 31, 33** and **35.**

** Except for **36,** which produces a very weak reaction.

within 2 sec for D3b and blue turning to brown within 2 sec for D3c. The detection limit is 0.5 $\mu g/cm^2$.

D4. The results are summarized in Table II.

D5. Only monohydroxy-monoalkoxy compounds (**25-33**) exhibit blue fluorescence. The detection limit is 0.4 $\mu g/cm^2$.

DISCUSSION

The results show that it is possible to differentiate the tetralins under study according to the substituents in their benzene and cyclohexene moieties.

Influence of aromatic ring substituents

D3a–f revealed the diphenols **34** and **35.** We assume that the diphenols are oxidized to the corresponding *p*-quinones by air in the presence of bases; the quinones then react with the amines. This is substantiated by the results of a model experiment. A quinone was prepared from **34** by oxidation with HIO_4. A red colour developed when 1-butylamine was added to the chloroform solution of the purified quinone, and gradually changed to orange. The latter dye was purified by chromatography on a silica gel column in chloroform and orange crystals were obtained on recrystallization from methanol. On TLC the sample gave one spot which exhibited an orange colour and fluorescence. When **35** was treated with D3b after spotting on the layer and chromatographed, the same spot was the major component. The same major spot was also obtained with **31** and **20** when treated with D2b after spotting (with **31** the heating after oxidation was not necessary).

D5 revealed the monophenolic monoalkoxy compounds **25–33** (not the monophenolics **37–39**). Dialkoxy compounds were not revealed using D3 or D5.

Substances without substituents in the aromatic ring were kindly supplied by Dr. I. Ivanov (Department of Organic Chemistry, Faculty of Pharmacy, Medical Academy, Sofia, Bulgaria). They included 2-hydroxyethylamino-3-hydroxytetralin, 2-carboxymethylamino-3-hydroxytetralin, 2-(N-ethyl-N-hydroxyethylamino)-3-hydroxytetralin and 2-(1-carboxyethyl)amino-3-hydroxytetralin. They were negative with all of the reagents.

Differentiation according to substituents in position 2

Primary and secondary 2-amino-3-hydroxy-5,8-dialkoxy compounds (and also the diol **23**) were differentiated from the remaining 5,8-dialkoxy substances by the colour reagents D2a–f and by the fluorescence reagent D4. Similar differences were noted among the 5- and 8-monohydroxy compounds, **37** contrasting with **38** and **39**. Selective reactions with D2a–f also occurred with 5,8-dihydroxy and 5,8-monohydroxy-monoalkoxy compounds carrying, next to the 3-hydroxy group, a hydroxy or primary or secondary amino group in position 2, *viz.*, **34, 25, 26** and **32**, in contrast to **35, 31** and **33.**

As stated in the introduction, the formation of quinones[5,6] and the oxidation of adjacent carbon atoms carrying an amino group (primary or secondary) in position 2 and a 3-hydroxy group[7,8] are expected, but we have not attempted to characterize the products of the reactions. (Reaction with D1 produces quinone derivatives even with the 5,8-dimethoxy compounds. It should be remembered that the concentration of HIO_4 increases during the heating owing to evaporation of water.)

According to refs. 7 and 8, vicinal primary or secondary alcohols are oxidized by HIO_4 or periodate to aldehydes. It is probable that the reactions of D2a–f and D4 with substances of type A in Table II proceed via dialdehydes. Substances of type B are unlikely to be cleaved by such an oxidation and they give negative reactions with D4 and yield colours with D2a–f different to those given by type A compounds. (Interestingly, when spots of type B compounds, after treatment with HIO_4 and heating, are sprayed with acetaldehyde and subsequently with butylamine, as in D2b, a green colour is obtained instead of brownish purple.) Oxidation of **23** with HIO_4 yielded a colourless compound [probably the dialdehyde, 1,4-dimethoxy-2,3-di(formylmethyl)benzene] which reacted with D2a–f and D4 as type A compounds. After treatment with a base (in solution or after spotting on a thin layer) this substance was converted into a multitude of fluorescent and coloured products which could be resolved chromatographically.

For the monomethoxy compound **36,** the reactions with D2a–f were very weak.

Other reactions for phenols

Other reactions for phenolic groups are also positive for the 5- and/or 8-hydroxy derivatives. The 4-nitrobenzenediazonium fluoroborate spray reagent exhibited low sensitivity for the 5,8-monohydroxy-monoalkoxy class (**25–33,** pink). Its sensitivity for the 5- or 8-monohydroxy class was satisfactory (**37–39,** orange).

Blue spots with the iron(III) chloride–hexacyanoferrate(III) reagent were sufficiently sensitive for the dihydroxy and monohydroxy-monoalkoxy compounds, yet preliminary tests with biological materials (microsomal incubates, urine extracts, etc.)

showed strong interference of other constituents of the sample. Reaction with D5, on the other hand, was specific and allowed the detection of low concentrations of **25** in the incubates.

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