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CHARACTERISATION OF OXIDATION PRODUCTS OF RAUWOLFIA ALKALOIDS

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The oxidation products of three Rauwolfia alkaloids having yohimbane skeleton, namely, reserpine, rescinnamine and ajmalicine have been characterised by chromatographic and spectroscopic techniques. The R_f values of the oxidation products (3, 4-dehydro derivatives) in several solvent systems and the spectral data on UV, IR, NMR and fluorescence characteristics have been determined to confirm the structure of these compounds.

Keywords: Rauwolfia alkaloids, rescinnamine, ajmalicine.

INTRODUCTION

It is well known that Rauwolfia alkaloids with yohimbane skeleton, namely, reserpine, rescinnamine and ajmalicine (antihypertensive agents) are unstable compounds and are oxidised by air and light (British Pharmacopoeia, 1993; United States Pharmacopeia, 1995; Cocolas, 1998; Parfitt, 1999). Upon standing most of the solutions of reserpine and rescinnamine acquire colour and pronounced fluorescence, especially after the addition of acid and on exposure to light (Fleckenstein, 2000; O'Neil, 2001). The oxidation of reserpine (Haycock *et al.*, 1966; Wright and Tang, 1972; Ahmad *et al.*, 1989); rescinnamine (Missan *et al.*, 1960; Joly and Bucourt, 1960), and ajmalicine (Wenkert and Roychaudhri, 1956) has been studied. Most of these studies have been carried out for preparative or analytical work involving the oxidation products.

The present work has been undertaken to identify the oxidation products of reserpine, rescinnamine and ajmalicine tentatively on the basis of chromatographic and spectral data, viz., R_f values and UV, IR, NMR and fluorescence characteristics.

MATERIALS AND METHODS

Reserpine was obtained from Sigma Chemical Co.; rescinnamine and ajmalicine were obtained from Ciba. Reserpine was recrystallised from methanol-chloroform (1:1, v/v) and rescinnamine and ajmalicine from methanol. 3,4-Dehydroreserpine, 3,4-dehydrorescinnamine and 3,4-dehydroajmalicine were prepared according to the procedures of Wright and Tang (1972), Joly and Bucourt (1960) and Wenkert and Roychaudhri (1956), respectively. All reagents and solvents were of the purest form available from BDH/Merck. The solvents were further purified by distillation before use.

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Thin-layer chromatography

Thin-layer chromatography (TLC) of the 3,4-dehydro products was carried out on 250-µm silica gel GF precoated plates using the following solvent systems:

- S_1: Methanol-chloroform (1:4, v/v) (Wright and Tang, 1972).
- S_2: n-Butanol-butanone-water (65:25:25, v/v) (Hakkesteegt, 1970).
- S_3: Chloroform-methanol (93:07, v/v) (Frijns, 1971).
- S_4: Carbon tetrachloride-methanol (96:4, v/v) (Hakkesteegt, 1970).
- S_5: Acetone-methanol-glacial acetic acid (70:25:05, v/v) (Court and Habib, 1973).
- S_6: n-Butanol-water-glacial acetic acid (4:1:1, v/v) (Bonati and Pesce, 1966).

The spots were located under UV light (350 nm).

Ultraviolet and visible spectrometry

All absorbance measurements and spectral determinations were made on a Shimadzu UV-visible recording spectrophotometer (model UV-240) using matched silica cells of 10 mm pathlength.

Infrared spectrometry

The infrared spectra (KBr disc) were obtained with a Jasco IRA-1 infrared spectrophotometer.

Nuclear magnetic resonance spectrometry

The ^{1}H-NMR spectra were recorded on Bruker AM-400 NMR spectrometer operating at 300 or 400 MHz, respectively. The abbreviation used for NMR data are as follows: s, singlet; d, doublet; dd, double doublet; ddd, triple doublet; t, triplet; br, broad; m, multiplet.

Fluorescence spectrometry

Fluorescence measurements were carried out at room temperature with a double beam Shimadzu RF 500 LC spectrofluorimeter. The fluorescence intensity scale was calibrated using 0.1 M quinine solution as standard (λ_{ex} 350 nm, λ_{em} 450 nm). The solutions were protected from light with aluminium foils to prevent photodegradation during handling.

RESULTS AND DISCUSSION

The oxidation products (i.e., 3,4-dehydro derivatives) of reserpine, rescinnamine and ajmalicine were identified by TLC using solvent systems, S_1-S_6, and comparing their R_f values with those of the authentic compounds. The spot fluorescence and R_f values of the oxidation products and the parent alkaloids are given in table-1. The R_f values of all these compounds are almost of the same magnitude as reported by earlier workers (Schlemmer and Link, 1959; Hakkesteegt, 1970; Frijns, 1971; Wright and Tang, 1972; Court and Habib, 1973) in specific solvent systems. The reproducibility of the R_f values in solvent systems, S_1-S_6, determined under controlled conditions, is of the order of ± 2%.

In order to confirm the structures of the 3,4-dehydro derivatives of reserpine, rescinnamine and ajmalicine, the UV, IR, NMR and fluorescence spectra were obtained. The UV (λ_{max}, log ε) and fluorescence (λ_{ex}, λ_{em}) characteristics of the compounds were determined and the IR and NMR structural assignments were made. The various spectral data on the 3,4-dehydro products along with those of the parent compounds are reported in table-2.

Table 1

R_f values of Rauwolfia alkaloids and their oxidation products*

Solvent System	Reserpine	3,4-Dehydro-reserpine	Rescinnamine	3,4-Dehydro-rescinnamine	Ajmalicine	3,4-Dehydro-ajmalicine
S_1	85	64	80	54	95	62
S_2	80	50	79	50	85	60
S_3	56	10	60	20	80	12
S_4	55	06	46	10	78	24
S_5	89	50	90	49	60	32
S_6	78	45	77	46	70	36

*R_f x 100

Spot fluorescence:

apple green (3,4-dehydroreserpine)
brownish green (3,4-dehydrorescinnamine)
yellow green (3,4-dehydroajmalicine)

Table 2

Spectral data on Rauwolfia alkaloids and their oxidation products

Compound	UV	IR	NMR	Fluorescence
Reserpine	λ_{max}, nm (log ε) (CH_3OH) 267 (4.195), 296 (3.984) (Sakai and Shellard, 1955).	ν_{max} (KBr, cm^{-1}) 3400, 1720, 1700, 1265, 1240 & 1220 (Moffat, 1986).	$CDCl_3$, (400MHz), δ ppm 7.33 (d, J=8 Hz, H-9), 7.70 (br, s, -NH), 7.30 (s, benzoyl ring proton), 6.78 (d, J=8Hz, H-10),6.84 (s, H-12), 3.99 (s, -OCH_3 proton), 4.48 (br s, H-3), 3.17 (m, H-5), 1.81 (ddd, H-14) (Lounasmaa and Tolvanen, 1988).	λ_{ex}, nm λ_{em}, nm
3,4-Dehydro-reserpine	(CH_3OH) 258 390 (4.386)	2940, 2850 (CH stretching) 1715 (C=O), 1635 (C=N), 1600, 1580 (C=C), 1220 (C-O).	7.96 (d, J=8 Hz, H-9), 7.40 (d, J=8 Hz, H-12), 7.35 (s, benzoyl ring proton), 7.0 (d, J=8 Hz, H-10), 3.99 (s,-OCH_3 proton), 4.20 (m, H-5), 3.20 (ddd, H-14).	acidified ethanol (pH ~ 2) 390 510 (Froehlich, 1981)
Rescinn-amine	(CH_3OH) 228 (4.788), 302 (4.475) (Sakai and Shellard, 1955).	ν_{max} (Nujol,cm^{-1}) 3390 (-NH), 1720, 1695 (ester), 1615, 1590, 1490 (C=C), 1233 (C-O). (Sakai and Shellard, 1955).	4.48 (br, s, H-3), 6.4 (d, J=17 Hz, cinnamic proton), 7.62 (d, J=17 Hz, cinnamic proton), 7.3 (d, J=7.3 Hz, H-9), 6.78 (d, J=8.9 Hz, H-10), 7.8 (s, -NH), 3.92 (s, ester), 6.85, (s, H-12), 3.18, (m, H-5), 2.96 (d, H-6), 1.79 (ddd, H-14) (Lounasmaa and Tolvanen, 1988).	

Table-2 contd.

Compound	UV	IR	NMR	Fluorescence
3,4-Dehydro-rescinn-amine	(CH_3OH) 268 388 (4.359)	2940, 2850 (CH stretching) 1715 (C=O), 1630 (C=N), 1615, 1600 (C=C), 1220 (C-O).	6.4 (d, J=15 Hz, cinnamic proton), 7.6 (J=15 Hz, cinnamic proton), 8.0 (d, J=7.8 Hz, H-9), 7.1 (d, J=9.0 Hz, H-10), 7.5 (s, H-12), 4.3 (m, H-5), 3.1 (ddd, H-14).	acidified ethanol (pH ~ 2) 388 510 (Froehlich, 1981).
Ajmalicine	(CH_3OH) 226 (4.647) 291 (3.826) (Sakai and Shellard, 1955).	3400, 1720, 1615, 1590, 1490, 1220. (Sakai and Shellard, 1955).	6.50 (d, J=7.5 Hz, H-9), 6.80 (d, J=8 Hz, H-12), 6.4 (t, J=8.5 Hz, H-10), 6.45 (t, J=10 Hz, H-11), 3.8 (s, ester proton), 3.6 (m, H-5), 3.2 (ddd, H-14), 3.0 (dd, H-21).	
3,4-Dehydro-ajmalicine	(CH_3OH) 284, 354 (3.410)	(Nujol,cm^{-1}) 3130 (CH stretching), 1635 (C=N), 1590, 1570 (C=C), 1540, 1220.	3.81 (s,-$COOCH_3$) 7.08 (t, J=8 Hz, H-10), 7.20 (t, J=10 Hz, H-11), 7.29 (d, J=8 Hz, H-9), 7.42 (d, J=8 Hz, H-12), 4.2 (m, H-5), 3.2 (dd, H-14), 3.5 (ddd, H-21).	

There are some distinct differences (e.g., λ_{max}, δ values) between the spectral characteristics of the oxidation products and their parent compounds (table-2). This may be due to structural variations resulting from oxidation of the molecules. The oxidation products exhibit characteristic fluorescence and may be distinguished from the parent compounds (non-fluorescent) on TLC plates. Thus, on the basis of the TLC and spectral data, the identity of the oxidation products of reserpine, rescinnamine and ajmalicine has been confirmed.

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