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CHEMICAL CONFIRMATION FOR THE CONFIGURATIONS ASSIGNED TO THE INDOLE ALKALOIDS, SPECIOGYNINE, SPECIOCILIATINE, MITRACILIATINE AND HIRSUTINE

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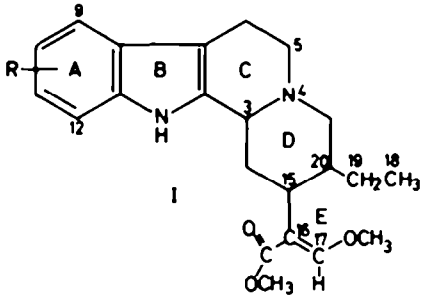
Abstract—The faster rate of reaction with mercuric acetate of speciogynine compared with mitraciliatine, confirms the assignment of the *normal* configuration to speciogynine. The assigned configurations of speciociliatine (*epiallo*), mitraciliatine (*pseudo*) and hirsutine (*pseudo*) are confirmed by formation of these alkaloids from mitragynine (*allo*), speciogynine (*normal*) and dihydrocorynantheine (*normal*), respectively, by mercuric acetate oxidation followed by zinc/acetic acid reduction.

THE configuration of mitragynine (I, R = 9-OCH_3), an indole alkaloid of the corynantheidine-type (I, R = H) has been established as *allo*,† with a *trans* (methoxy/carbomethoxy) C16-C17 double bond geometry, by X-ray crystallography.[1]



I

The assignment of configuration to the three diastereomers of mitragynine, viz. speciogynine, speciociliatine and mitraciliatine,[2-4] rests on the assignment of certain spectral parameters (IR and NMR) to the preferred conformation of a given configuration.[5,6] Independent confirmation of these assignments is desirable because it would support the preferred conformation allocated to a given configuration[5,6] and

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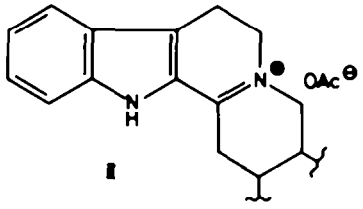
† Terminology for the four possible configurations is as follows:

configuration	C3H	C15H	C20H
normal	α	α	α
pseudo	β	α	β
allo	α	α	α
epiallo	β	α	α

would permit the use of these spectral criteria in determining the configuration of similar alkaloids. A possible and attractive method for establishing the configurations of these alkaloids by chemical means was oxidation of the appropriate alkaloid with mercuric acetate followed by reduction with zinc in acid. Mercuric acetate oxidises alkaloids of the yohimbine[7] and ajmalicine[8] type to yield the unsaturated salt intermediates with basic skeletal structure II. Subsequent reduction of II with Zn/HCl yields the C3 epimer of the starting alkaloid.[7,8]



II

Mechanistically the oxidative reaction requires a coplanar (diaxial) attack of the mercuric acetate anion.[9] Hence an alkaloid which has both the C3H and the N4 lone pair of electrons *axially* orientated in its preferred conformation will react much faster with mercuric acetate than will an alkaloid which has either the C3 or the N4 lone pair *equatorially* orientated in its preferred conformation. Under identical conditions, mitragynine reacted more quickly with mercuric acetate than speciociliatine, speciogynine more quickly than mitraciliatine and dihydrocorynantheine than hirsutine.

Treatment of mitragynine (I, *allo*, R = 9-OCH_3) with mercuric acetate followed by Zn/HOAc reduction yielded a mixture which was shown by TLC to consist predominantly of two components, mitragynine and another compound which upon isolation was found to be identical (equiv wt, TLC, UV, IR, NMR, ORD, CD) with speciociliatine (I, R = 9-OCH_3). Speciociliatine, therefore, must have the *epiallo* configuration, a conclusion which is in agreement with that previously deduced by spectral means.[6]

The mitragynine produced by reduction of the unsaturated amine salt intermediate was identical with natural mitragynine (UV, IR, NMR, ORD, CD) and therefore no isomerisation occurred about the C16–C17 double bond, under the reaction conditions. Hence speciociliatine, in accord with mitragynine, can be assigned the *trans* (methoxy/carbomethoxy) geometry about the C16–C17 double bond (see also Ref. 6).

The identical ORD and CD curves given by "natural" speciociliatine and "synthetic" speciociliatine establish that mitragynine and speciociliatine have similar absolute configurations. The absolute configuration of mitragynine[1,6] is known to be C3Hα, C15Hα and C20Hα, and therefore the absolute configuration of speciociliatine must be C3Hβ, C15Hα, and C20Hα. This conclusion is in agreement with that reached previously and indicates, as postulated,[6] the sign of the Cotton effect in the 270–300 mμ region can be correlated with the stereochemistry at C3 in alkaloids of the corynantheidine-type (I).

Of the two remaining diastereomers (I, R = 9-OCH_3), speciogynine and mitraciliatine, one must have the *normal* configuration and the other the *pseudo* configuration. The preferred ring D chair conformations for the *normal* configuration is undoubtedly that conformation in which the C/D ring junction is *trans* fused while

the C15 and C20 substituents are *equatorial*,[5,6] thus orientating the C3H and N4 lone pair in *axial* positions, ideal for attack by mercuric acetate and acetate anion. The preferred ring D chair conformation for the *pseudo* configuration is probably that conformation in which the C/D junction is *cis* fused, placing the C3H *equatorial* while the C15 and C20 substituents are *equatorial*. The alternate ring D chair conformation in which the C/D ring junction is *trans* fused would require the C15 and C20 substituents to be *axial*. Thus it is safe to assume that the rate of mercuric acetate reaction for an alkaloid of the *normal* configuration will be faster than that for an alkaloid of the *pseudo* configuration. Under identical conditions, speciogynine reacted with mercuric acetate more quickly than mitraciliatine, and therefore speciogynine can be assigned the *normal* configuration and mitraciliatine the *pseudo* configuration in agreement with the configurations assigned earlier.[6]

Treatment of speciogynine with mercuric acetate followed by Zn/HOAc reduction yielded a mixture which was shown by TLC to consist predominantly of two components, speciogynine and another compound which on isolation was found to be identical (m.p., equiv wt, TLC, UV, IR, NMR, ORD, CD) with mitraciliatine. The identical ORD and CD curves of "natural" mitraciliatine and "synthetic" mitraciliatine establishes that speciogynine and mitraciliatine have similar absolute configurations. Therefore the absolute configuration of speciogynine is C3Hα, C15Hα, C20Hβ and that of mitraciliatine is C3Hβ, C15Hα and C20Hβ (see also Refs 5 and 6).

The configurations of dihydrocorynantheine (I, R = H) and corynantheidine (I, R = H) have been well established as *normal*[10-12] and *allo*[10,11,13] respectively. Of the two remaining configurational possibilities, i.e. *pseudo* and *epiallo*, left for an alkaloid possessing this structure, the *pseudo* configuration has been assigned to hirsutine,[14] on the basis of spectral data.[15] Oxidation of dihydrocorynantheine with mercuric acetate followed by Zn/HOAc reduction yielded a mixture which was shown by TLC to consist predominantly of two components with hR_f values corresponding to dihydrocorynantheine and hirsutine on four TLC systems. Similar treatment of corynantheidine yielded a mixture which was shown by TLC to consist predominantly of two components, one with hR_f values corresponding to corynantheidine and the other with hR_f values different from those of hirsutine on four TLC systems. Hirsutine must therefore be assigned the *pseudo* configuration.

EXPERIMENTAL

All m.ps are uncorrected. Equiv wts were determined by non-aqueous titration using N/50 perchloric acid in glacial HOAc and oracet blue as indicator. All UV, IR, NMR, ORD and CD spectra were measured as previously described.[6] Adsorbent for column chromatography, Spence type H alumina; adsorbents for TLC, silica gel G (Merck) and alumina G (Merck). TLC systems used: (a) alumina–$CHCl_3$; (b) alumina-$CHCl_3$:cyclohexane (7:3); (c) silica gel-$CHCl_3$:acetone (5:4); (d) silica gel-benzene:EtOAc:Et_2NH (7:2:1).

Mercuric acetate oxidation of mitragynine, speciociliatine, speciogynine, mitraciliatine, dihydrocorynantheine and hirsutine

The concentrations used were based on those previously described.[16] The alkaloid (10 mg) was dissolved in 10% mercuric acetate in 5% HOAc (0·5 ml), continually stirred with a glass rod for 30 min at 60° and the time of formation of mercurous acetate ppt was noted. A heavy white crystalline ppt formed with mitragynine (2 min), speciogynine (10 min) and dihydrocorynantheine (14 min); dark yellow solns remained after the removal of mercurous acetate. The resulting solns from speciociliatine, mitraciliatine and hirsutine

were a paler yellow and no ppt of mercurous acetate formed after 30 min. Mercuric ions were removed by addition of thioacetamide, the filtered solns made alkaline with conc NH_4OH, extracted with $CHCl_3$ and concentrated. The concentrates were examined by TLC systems (a), (b), (c) and (d), the results showing that only traces of mitragynine, speciogynine and dihydrocorynantheine were present in their respective reaction mixtures whilst speciociliatine, mitraciliatine and hirsutine were major components of their respective reaction mixtures. hR_f values:

mitragynine	(a) 97, (b) 76, (c) 73, (d) 85
speciogynine	(a) 83, (b) 63, (c) 56, (d) 83
dihydrocorynantheine	(a) 95, (b) 72, (c) 63, (d) 84
speciogynine	(a) 53, (b) 40, (c) 21, (d) 77
mitraciliatine	(a) 34, (b) 22, (c) 8, (d) 59
hirsutine	(a) 43, (b) 30, (c) 9, (d) 62

Isomerization of mitragynine

Mitragynine (2·0 g) was dissolved in glacial HOAc (50 ml) containing mercuric acetate (2·4 g) and heated at 60° for 18 hr. The ppt of mercurous acetate was filtered off, H_2S passed into the filtrate to remove mercuric ions, filtered, powdered Zn (5 g) added to the filtrate and stirred for 20 hr. TLC indicated that further reduction was required. Conc HCl (20 drops) and powdered Zn (5 g) added and stirring continued for a further 48 hr. After filtration, the filtrate was made alkaline with conc NH_4OH, extracted with ether which was washed, dried and concentrated to a cream coloured amorphous solid (1·7 g). Three separate portions (500 mg) were dissolved in ether (1 ml), added to an alumina column (20 cm in length, 1 cm diam) and eluted with dry ether. The eluate was automatically fractionated, each fraction (5 ml) being examined by TLC, like fractions being combined and evaporated to dryness. The two major compounds isolated were:

(i) a white amorphous solid (400 mg), identical to speciociliatine, obtained from *Mitragyna speciosa*, in equiv wt, UV, IR, NMR, CD, ORD spectra[2, 3, 6] and hR_f values in TLC systems (a), (b), (c) and (d).

(ii) a colourless crystalline solid (550 mg) from absolute EtOH, identical to mitragynine in m.p., mixed m.p., UV, IR, NMR, CD, ORD spectra[2, 3, 6] and hR_f values in TLC systems (a), (b), (c) and (d).

Isomerization of speciogynine

Speciogynine (1·36 g) was dissolved in glacial HOAc (25 ml) containing mercuric acetate (1·63 g) and heated at 60° for 48 hr. The ppt of mercurous acetate was filtered off, H_2S passed into the filtrate to remove mercuric ions, filtered, powdered Zn (4 g) and water (10 ml) added to the filtrate which was stirred for 10 hr. After filtration, the filtrate was made alkaline with conc NH_4OH, extracted by $CHCl_3$ which was washed, dried and concentrated to a brown amorphous solid (1·23 g). The solid was dissolved in ether (1 ml), added to an alumina column (10 cm in length, 2·5 cm diam) and eluted with ether (100 ml) followed by $CHCl_3$ (100 ml). The ethereal eluate was concentrated (to 10 ml) and on standing yielded a white crystalline solid (0·63 g) identical in m.p., mixed m.p., UV, IR, NMR, CD, ORD spectra[2, 3, 6] and hR_f values in TLC systems (a), (b), (c) and (d) with speciogynine. The $CHCl_3$ eluate was concentrated to dryness yielding a cream solid (0·22 g) which was dissolved in ether (1 ml), added to an alumina column (5 cm in length, 1 cm diam) and eluted with dry ether (300 ml). The ethereal eluate was concentrated to dryness (120 mg), dissolved in dry ether (10 ml), yielding a white crystalline solid (68 mg), identical in m.p., equiv wt, UV, IR, NMR, CD, ORD spectra[4, 6] and hR_f values in TLC systems (a), (b), (c) and (d) to mitraciliatine previously isolated from *Mitragyna ciliata*[4] NMR spectrum in $CDCl_3$ at 60 Mc in ppm (δ) from TMS:

Three-proton triplet	0·80	(CH_2CH_3)
Three-proton singlet	3·68	(OCH_3)
Three-proton singlet	3·73	(OCH_3)
Three-proton singlet	3·89	(OCH_3 aromatic)
One-proton multiplet	4·45	(C3H, N4 cis)
One-proton multiplet	6·52	(aromatic)
Two-proton multiplet	7·03	(aromatic)
One-proton singlet	7·33	(olefinic)
One-proton singlet	8·05*	(imino)

* Disappears on deuteration

Isomerization of corynantheridine and dihydrocorynantheine

The alkaloid (15 mg) was dissolved in glacial HOAc (5 ml) containing mercuric acetate (20 mg) and heated at 60° for 2½ hr, filtered and stirred with Zn powder (100 mg) for 2 hr. After filtration, the filtrate was made alkaline with conc NH_4OH, extracted with ether which was washed, dried and concentrated (to 0·5 ml). TLC showed the following major components, other than starting material:

isomerization of corynantheidine	(a) 57, (b) 55, (c) 23, (d) 79
isomerization of dihydrocorynantheine	(a) 43, (b) 30, (c) 9, (d) 62
hirsutine as reference	(a) 43, (b) 30, (c) 9, (d) 62

There was no spot in the corynantheidine isomerization mixture corresponding to hirsutine.

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