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GARDNERIA ALKALOIDS - VI1)

STRUCTURES OF GARDNERAMINE AND ALKALOID G (DEMETHYLGARDNERAMINE)

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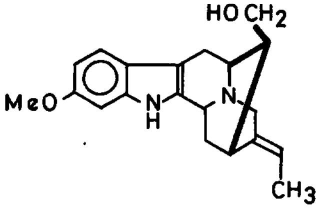
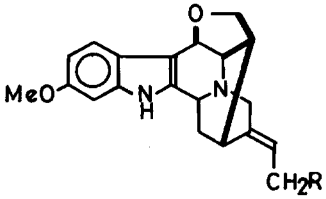
Our previous papers described the isolation of four new indolic alkaloids from Gardneria nutans Sieb. et Zucc. (Loganiaceae)2) and the structural studies which allowed us to establish the following structures for gardnerine, gardnutine and hydroxygardnutine.3,4)



GARDNERINE



GARDNUTINE (R=H)

HYDROXYGARDNUTINE (R=OH)

The present communication is concerned with the elucidation of structural formula (I) for the fourth base, gardneramine (I), $C_{23}H_{28}N_2O_5$, mp 134-135°, $[\alpha]_D$ -287.7°.

The NMR spectrum[*1] of I revealed the presence of four methoxyl groups, three being aromatic (τ 6.05, 6.10 and 6.15) and one aliphatic (τ 6.64). The remaining oxygen must be forming an ether ring, for I showed no OH or carbonyl absorption in its IR spectrum. A $>C{=}N-$ system was suggested by a strong IR absorption at 1590 cm^{-1}, while the other nitrogen was postulated to be tertiary by the fact that I failed to give any N-acyl derivative and by the absence of NH absorption in its IR and NMR spectra. The presence of a tri-substituted double bond was evident from an olefinic proton in the NMR spectrum at τ 4.61 (t.t., J=7.3 and 2.5 Hz). The UV spectrum of I (λ_{max} 264(3.82), 270(3.80) and 324(3.78) mμ) was of little diagnostic value, because no similar curve was found in the literature.

Heating I with 85 % formic acid gave a powder (II), $C_{23}H_{30}N_2O_6$, λ_{max}253(sh) and 314 mμ, which showed the absorption bands due to the newly formed carbonyl (1730 cm^{-1}), NH(3425 cm^{-1}) and OH (3300-3200 cm^{-1}) groups in its IR spectrum. Acetylation of II afforded O-acetate (III),

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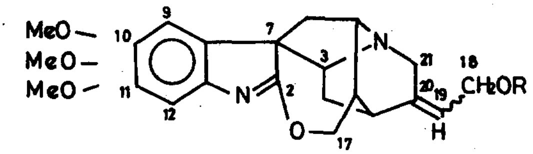
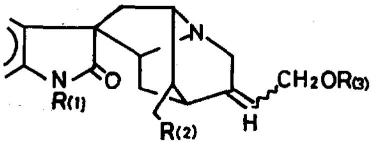
[*1] : All NMR spectra were measured at 100 MHz in $CDCl_3$ with TMS as the internal standard.



GARDNERAMINE (I) R= CH_3

ALKALOID G ≡ (X) R= H



	$R_{(1)}$	$R_{(2)}$	$R_{(3)}$		$R_{(1)}$	$R_{(2)}$	$R_{(3)}$
II	H	OH	CH_3	VI	CH_3	H	CH_3
III	H	OAc	CH_3	VII	H	Cl	CH_3
IV	H	OTs	CH_3	VIII	H	Cl	H
V	CH_3	I	CH_3	IX	H	Cl	Ac

$C_{25}H_{32}N_2O_7$, which was also formed by direct acetolysis of I in boiling AcOH. A mild treatment of II-tosylate (IV), $C_{30}H_{36}N_2O_8S$, mp 158-165°, with ethanolic KOH regenerated the starting base (I) in an excellent yield. On attempted reduction of II using $NaBH_4$ in boiling EtOH, the unchanged material was recovered. From the above chemical evidences and characteristic spectral data, it was concluded that II has an oxindole moiety which was generated from a hydrolytic cleavage of the ether ring of I as shown below.

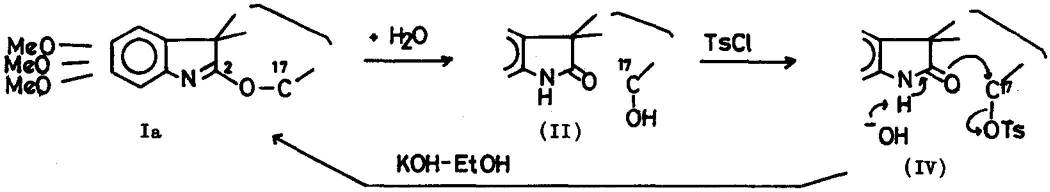
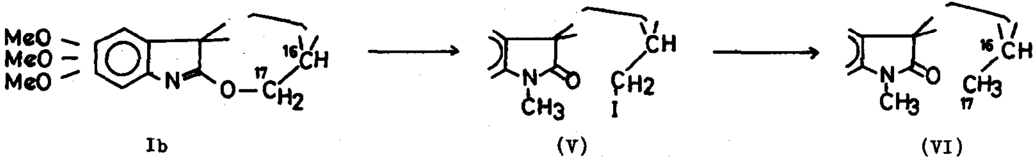


Chart 1.

The NMR signal due to $C_{(17)}$-methylene of II appeared as a doublet (J=7.5 Hz) at τ 6.02, which exhibited downfield shifts to τ 5.55 (d. J=8.7 Hz) in acetate (III) and to τ 5.52 (d. J=8.7 Hz) in tosylate (IV) respectively. Reaction of I with CH_3I in dry benzene afforded an N-methylated oxindole (V), $C_{24}H_{31}N_2O_5I$, mp 143-145°, τ 6.64 (3H,s. N-Me), together with two uncaracterized quarternary salts. A reductive dehalogenation using Zn-AcOH gave a halogen-free compound (VI), which showed the expected C-Me signal at τ 8.79 as a doublet (J=7.5 Hz) in its NMR spectrum. Therefore the partial structure can now be extended to Ib.

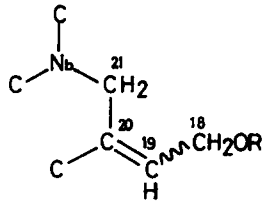


Refluxing I with dilute HCl yielded two chlorine-containing oxindoles (VII), $C_{23}H_{29}N_2$-

O_5Cl, and (VIII), $C_{22}H_{27}N_2O_5Cl$, mp 110-118°, both of which showed the characteristic signals due to $C_{(17)}$-methylene, adjacent to Cl, as doublets at 6.02 (J=8.0 Hz)(VII) and τ 6.03 (J=8.0 Hz) (VIII), in their NMR spectra. Comparison of their molecular formulae and NMR spectra showed that VIII is the demethylation product of VII resulted from a hydrolytic loss of an allylic methoxyl group. In fact, treatment of VII with dilute HCl afforded VIII, and the latter gave an O-acetate (IX) on usual acetylation. The NMR data tabulated below and the above evidences indicate the presence of the following system (Ic).

Table 1. NMR data (Chemical shifts in τ values)

	$C_{(18)}H_2$	$C_{(19)}H$	$C_{(21)}H_2$	$C_{(18)}OR$
I ($R=CH_3$)	(6.10)*2	4.61 t.t. J=7.3, 2.5 Hz	(6.30)*2	6.64 3H, s.
VII ($R=CH_3$)	(6.17)*2	4.68 t.t. J=6.7, 2.0 Hz	(6.25)*2	6.69 3H, s.
VIII (R= H)	5.92 d. J=6.7 Hz	4.64 t.t. J=6.7, 2.0 Hz	(6.26)*2	8.20 1H, s.
IX (R=Ac)	5.50 d. J=7.5 Hz	4.68 m.	(6.24)*2	7.95 3H, s.



Ic

Gardneramine (I) was regenerated from VII on treatment with ethanolic KOH in the same manner as with IV (c f. Chart 1). An analogous base catalyzed dehydrochlorination of VIII afforded X, and the latter was found to be identical with an unknown base, alkaloid G, which had been isolated from G. multiflora Makino in this laboratory[5]. Thus the correlation of alkaloid G with gardneramine (I) has been established.

Von Braun reaction of I yielded cyanobromide (XI), $C_{24}H_{28}N_3O_5Br$, mp 214° and it showed the identical UV curve with that of I, indicating the reaction took place at $N_{(b)}$ not at $N_{(a)}$. The cleavage of the imino-ether ring of XI was successfully made by use of CH_3I to give a $N_{(a)}$-methylated oxindolic product (XII), m/e 659 and 661 (M^+ and M^++2, $C_{25}H_{31}N_3O_5BrI$), λ_{max}263 and 316 mμ, ν 2205 (−C≡N) and 1690 (oxindolic C=O) cm^{-1}. This observation demonstrated that no participation of $N_{(b)}$ is required for the formation of the oxindole system, supporting the proposed partial structure (Ib).

All the above and other chemical evidences and spectral data are compatible with the structural formula (I) of gardneramine. In addition, mass spectrometric study provided another

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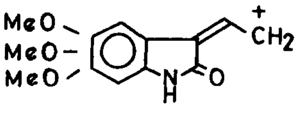
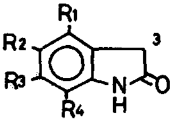
*2 : The corresponding signal is obscure because of overlapping with other signal(s). The chemical shift value in the parenthesis was determined by the double resonance experiments.

support for the skeletal structure. Thus a characteristic fragment of m/e 248 was observed in the mass spectra of all the oxindolic derivatives II - IX except N-methylated derivatives (V) and (VI), in which the corresponding peak appeared at m/e 262 (248 + CH_2). High resolution mass spectrometric determination gave the composition $C_{13}H_{14}NO_4$ (m/e 248), which can be depicted as shown. This type of fragmentation is characteristic to the oxindole alkaloids having a quinuclidine system in the alicyclic moiety.$^{6)}$



m/e 248

A degradation product (XVII), mp 100-101°, λ_{max} 257 and 306 mμ, ν 3420 (oxindolic N-H) and 1714 (oxindolic C=O) cm^{-1}, was obtained by Zn dust distillation of I or III, and its structure was found to be 3-ethyloxindole having three methoxyl groups on the benzene ring. Compounds (XIII), (XV) and (XVI) were synthesized for comparison, but none of the UV spectra agreed with that of XVII. Accordingly, the degradation product (XVII) is considered to be 3-ethyl-XIV, and the methoxyl groups are probably situated at $C_{(9)}$, $C_{(10)}$ and $C_{(12)}$ of the benzene ring of gardneramine (I). All the efforts have not been successful in synthesizing XVII or XIV, so far.



	R_1	R_2	R_3	R_4
XIII	OCH_3	OCH_3	OCH_3	H
XIV	OCH_3	OCH_3	H	OCH_3
XV	OCH_3	H	OCH_3	OCH_3
XVI	H	OCH_3	OCH_3	OCH_3

A discussion on the remaining structural features of gardneramine (I), including the configuration of the $>C_{(19)}=C_{(20)}<$ system and the absolute configuration of the molecule will be made in the accompanying communication, which deals with the X-ray crystallographical study of the structure of cyanobromide (XI).

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