Phytochemistry, Vol. 27, No. 6, pp. 1849–1852, 1988.
Printed in Great Britain.

0031-9422/88 $3.00 + 0.00
Pergamon Press plc.

SHORT REPORTS

ISOCRAUGSODINE, AN *N*-ARYLIDENEPHENETHYLAMINE FROM *CRINUM ASIATICUM* AND ITS *E–Z* ISOMERISM*

SHIBNATH GHOSAL, AMIRTHALINGAM SHANTHY and SUSHIL K. SINGH

Pharmaceutical Chemistry Research Laboratory, Department of Pharmaceutics, Banaras Hindu University, Varanasi 221005, India

(*Received* 4 *September* 1987)

Key Word Index—*Crinum asiaticum*; Amaryllidaceae; *N*-(3-methoxy-4-hydroxybenzylidene)-4′-hydroxyphenethylamine; isocraugsodine; *E–Z* isomerism.

Abstract—A new Schiff's base, named isocraugsodine, was isolated from the fruits of *Crinum asiaticum*. Its *N*-(3-methoxy-4-hydroxybenzylidene)-4′-hydroxyphenethylamine structure was assigned on the basis of chemical transformation and comprehensive spectroscopic evidence. The temperature-gradient distribution of the three isomeric forms (**1a**⇌**1b**⇌**1c**) of the Schiff's base was determined by high resolution ^{1}H NMR analysis. Isocraugsodine is considered as a direct precursor to Amaryllidaceae alkaloids.

INTRODUCTION

Lycorine, the first member of the Amaryllidaceae alkaloids, was isolated in 1877. Since that time, there has been no lack of interest in the chemistry and biology of this group of compounds [1] although only cursory attention has been paid to the occurrence and stability of their first advanced precursors—the *N*-arylidene-4-hydroxyphenethylamines. In a recent communication, we reported the occurrence in *C. augustrm* Roxb. of such a Schiff's base which we named craugsodine. We provided indirect evidence in support of its isomeric structures (**2a**⇌**2b**⇌**2c**) in solution and considered its possibility as a direct precursor to Amaryllidaceae alkaloids [2]. In this paper, we report the occurrence of another Schiff's base, named isocraugsodine, in the fruits of *C. asiaticum* Linn. and its *E–Z* isomerism in solution.

RESULTS AND DISCUSSION

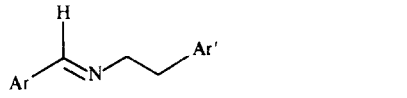
Semi-preparative HPLC of the methanol-soluble fraction from aq. methanol extracts of fruits of *C. asiaticum* afforded the new compound (yield, 0.0016%) along with a previously reported lignoid alkaloid, crinasiatine [3]. Characterization of the new compound only is described here.

Isocraugsodine

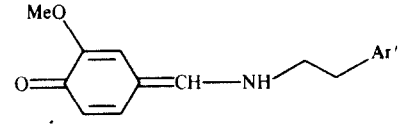
This compound, $C_{16}H_{17}NO_3$ (elemental analyses and M^+), mp 220°, showed UV spectrum, in methonal, and in the presence of acidic and basic shift reagents (see Experimental), characteristic of an *N*-arylidene-hydroxyphenethylamine containing an OH group *para* to the C=N bond. Its IR spectrum in Nujol, exhibited a strong twin peak at ν_{max} 1645 and 1648 cm^{-1}, characteristic of a vinylogous amide. On reduction of the C=N bond with sodium borohydride–methanol, it afforded an arylphenethylamine, $C_{16}H_{19}NO_3$. These properties suggested isocraugsodine as a Schiff's base.

Schiff's bases commonly assume two configurations (*E–Z* via the quinone methide) in solution. Such a possibility was considered before for craugsodine (**2a**⇌**2b**⇌**2c**) from indirect evidence [2]. Distinct separation of two of the three isomers (*E*-⇌quinone methide⇌*Z*-isomer) of craugsodine was discerned in its analytical HPLC in methanol–water (4:1). Attempts to separate the two isomers by semi-preparative HPLC, however, resulted in the convergence of the two peaks into a broad band. The energy difference of the two isomers of craugsodine seemed not to be sufficiently large to enable them to be recorded distinctly in the ^{1}H NMR time scale. In the 400 MHz ^{1}H NMR spectrum of craugsodine, in CD_3OD, at ordinary temperature (22°), considerable line broadening was observed in respect of the arylidene ring protons and H-7 signals. This was, presumably, due to overlapping of signals of the two isomeric species. When the temperature was raised to 60°, the broad signals were sharpened and revealed distinct multiplicities with appropriate *J* values, ascribable to the thermodynamically stable isomer. The 400 MHz ^{1}H NMR spectrum of isocraugsodine, on the other hand, provided cogent evidence in support of the existence of the three isomers (**1a**⇌**1b**⇌**1c**). Furthermore, temperature gradient distibution of the three isomers, in CD_3OD,

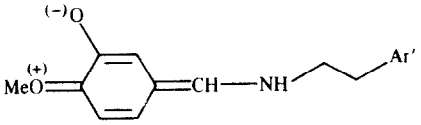
*Part 27 in the series: 'Chemical Constituents of Amaryllidaceae'. For Part 26 see ref. [8].



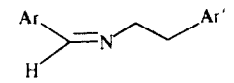
1a
2a



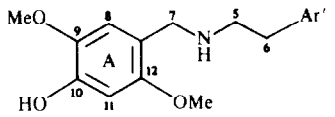
1b



2b



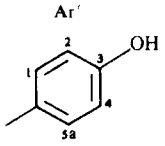
1c
2c



3

Ar

1a = **1c** = 3-Methoxy-4-hydroxyphenyl
2a = **2c** = 3-Hydroxy-4-methoxyphenyl



was determined by this method. The expanded Ar-methoxyl signals, due to the three isomers, were integrated at three temperatures to measure the percentage composition of each species (Table 1). Assignments of the other proton signals were also made in respect of the three species (Table 2). Earlier studies showed that Ar-unsubstituted aldimines exist almost completely (>99%) in the *E*-configuration [4]. However, the barrier to *E*–*Z* distribution, in aldimines, about the C–N double bond has been found to be very sensitive to the nature of the aromatic substituents. An OH group *para* to the C–N double bond would tend to stabilize the co-planar quinone methide [e.g. (**1b**)]. Consequently, at elevated temperatures, an appreciable contribution of this species (**1b**) was expected and realized (Table 2). The contribution of the *Z*-isomer (**1c**), though small at ambient temperature, increased perceptibly at higher temperatures.

The two major isomers (**1a** and **1b**) were subsequently separated by preparative TLC using chloroform–methanol (22:3) as developer. The two R_f zones at 0.65 and 0.74 were isolated and worked-up to give (**1a**) and (**1b**), respectively. These were separately dissolved in methanol and their UV spectra were determined quickly. The compound from the lower R_f zone exhibited UV λ_{max} 225, 271, and 304 nm, characteristic of the *E*-isomer, while the one from the upper R_f zone showed λ_{max}225 sh, 244 sh, 272, 310 and 398 nm, due to a mixture of the *E*-isomer and the quinone methide.

In the analytical HPLC of isocraugsodine, only two peaks, one sharp [due to the *E*-isomer (**1a**)] and one broad [due to overlapping of (**1b**) and (**1c**)] were discerned. The areas of the two peaks varied even with minor changes in the conditions of HPLC, eg. in the flow rate, sensitivity, and concentration of the applied solution. Attempts to separate the two major isomers by semi-prep. HPLC always ended up in a mixture of the three isomers (^{1}H NMR). However, immediately after dissolution of the *E*-isomer, from preparative TLC, only the signals due to (**1a**) were detected in the ^{1}H NMR analysis. After a few min., the resonances due to both (**1a**) and (**1b**) appeared and the concentration of the former decreased in concert.

The chemical proof for the *N*-(3-methoxy-4-hydroxybenzylidene)-4′-hydroxyphenethylamine structure of isocraugsodine was obtained by its conversion into a known alkaloid, belladine, and finally by its synthesis. Permethylation of isocraugsodine with excess of ether–methanol–diazomethane followed by reduction of the $C{=}\overset{(+)}{N}Me$ with sodium borohydride–methanol afforded belladine [6, 7]. Finally, condensation of vanillin and tyramine, in methanol, afforded the Schiff's base in a high yield (*ca* 90%). A minor side product (**3**), isolated from this reaction, suggested Michael-type addition of the solvent molecule (MeOH) to the quinone methide (**1b**). When the Schiff's base was refluxed for 1 hr, in MeOH, this product, 9-*O*-methyl-12-methoxynorbelladine (**3**),

Table 1. Percentage composition of the three isomers of isocraugsodine at different temperatures*

Isomer	OMe δ_H	Temperature (°):	Percentage composition at 20	40	60
1a	3.88		88.7	76.7	70.2
1b	3.90		9.4	21.0	26.5
1c	3.85		1.9	2.3	3.3

*From integration of expanded methoxyl signal in 400 MHz ^{1}H NMR spectrum in CD_3OD.

Table 2. ^{1}H NMR data of the three isomers of isocraugsodine*

Assignment of protons§	1a	1b Chemical shifts†	1c
H-1,5a	7.02 ((*d*, 9)	7.07 (*d*, 9)	‡
H-2,4	6.70 (*d*, 9)	6.74 (*d*, 9)	‡
CH_2-5	2.88 (*t*, 7)	2.75 (*t*, 7)	2.82 (*t*, 7)
CH_2-6	3.75 (*t*, 7)	2.96 (*t*, 7)	3.74 (broad)
H-7	7.92 (*s*)	7.68 (*s*)	8.10 (s)
H-8	7.35 (*d*, 2.5)	7.42 (*d*, 2)	‡
H-11	6.76 (*d*, 9)	6.88 (*d*, 8.5)	6.79 (*d*, 9)
H-12	7.08 (*dd*, 9, 2.5)	7.40 (*dd*, 8.5, 2)	7.11 (m)

*400 MHz ^{1}H NMR in CD_3OD

†In δ ppm from TMS at zero; multiplicities/J in Hz in parentheses.

‡Obscure (presumably buried under those of (**1a**).

§Arbitrary numbering taken from ref. [5].

was produced in appreciable amount. The identity of this compound was established by means of comprehensive spectroscopic analyses (see Experimental). The mixture of minor side products, after separation of (**3**), was methylated with Et_2O–CH_2N_2 and subjected to tandem MS analysis when oxomaritidine [2] was detected. Collision-induced dissociation for both M^+ and $[MH]^+$, at both high and low energy, was used to bring about the desired fragmentation (MS/ms).

The isocraugsodine is a native compound and not an artefact was established by analytical HPLC of cold EtOH extracts of *C. asiaticum* fruits when its presence was detected. This compound has not been encountered before in nature nor has it been chemically synthesized. The natural occurrence of Schiff's bases (1_{a-c}) and (2_{a-c}) and their facile conversion into a well known Amaryllidaceae alkaloidal ring system (oxomaritidine and equivalents) lend credence to our earlier postulate [2] that *N*-arylidene-4-hydroxyphenethylamines are the direct precursors to Amaryllidaceae alkaloids.

EXPERIMENTAL

Isolation procedure. The pericarp of fresh, mature fruits (*ca* 500 g) of *C. asiaticum* (5-year-old plant), collected in February 1984, from the Banaras Hindu University Campus, was macerated in MeOH; the mixture was warmed for 15 min at 60° then filtered. The filtrate was evapd *in vacuo* and the residue was triturated with hexane, CH_2Cl_2 and MeOH in succession. The residue from the MeOH-soluble fraction was dissolved in MeOH–H_2O–$CHCl_3$ (14:5:1) and passed through a 0.5 μm filter and then injected into a semi-prep. HPLC system [Spectra-Physics, RP-8 column, 440/254 nm detector, flow rate, 8 ml/min]. Fractions having retention time (R_t) between 12.5 and 13.8 min were collected. The process was repeated several times to obtain about 80 mg of isocraugsodine. It crystallized form MeOH as orange-yellow needles, mp 220°; R_t [Waters Associates analytical HPLC, μ Bondapak C_{18} column, MeOH–H_2O (4:1) as developer]: 6.2 min (broad), 7.8 min (sharp); λ_{max}(MeOH) nm (log ε) 225 (4.31), 271 (3.88), 304 (3.60), 398 (3.64); λ_{max}(MeOH–H_2SO_4)nm 225 sh, 244, 310 sh, 350; λ_{max} (MeOH–NaOMe)nm 225–232 sh, 282, 322; MS: *m/z* (rel int. %) 271 (M^+, 58), 164 (22), 163 (24), 135 (17), 121 (11), 107 (100). (Found: C, 70.52; H, 6.41; N, 5.05. $C_{16}H_{17}NO_3$ requires C, 70.8; 6.2; N, 5.1).

Reduction of isocraugsodine. Isocraugsodine (11 mg) in MeOH (92 ml) was reduced with $NaBH_4$ (24 mg). Usual work-up of the product afforded a gummy material which was dissolved in Et_2O–MeOH and chromatographed over a column of Florisil (20 g). Elution was carried out with $CHCl_3$, $CHCl_3$–MeOH (99:1, 19:1) and fractions (50 ml) were collected. The $CHCl_3$–MeOH (95:5) eluates afforded 9-*O*-methylnorbelladine as an amorphous solid (8 mg); R_t 4.87 min; MS: *m/z* 273 (27), 272 (38), 271 (18), 166 (21), 165 (14), 152 (18), 151 (26), 137 (100), 122 (9), 107 (40). (Found: C, 70.18; H, 6.77; N, 5.00. $C_{16}H_{19}NO_3$ requires C, 70.3; H, 6.9; N, 5.1).

Conversion of isocraugsodine into belladine. Isocraugsodine (18 mg), in MeOH (1 ml), was treated with a strong soln of Et_2O–MeOH–CH_2N_2 (20 ml) and the mixture was kept at room temp. overnight. The solvent was evapd and the residue was triturated with Et_2O. The Et_2O-insoluble solid, containing per-*O*-methyl-*N*-methylisocraugsodine cation, was dissolved in MeOH and reduced with $NaBH_4$ (40 mg). Usual work-up of the product afforded belladine which was further purified by prep. TLC. It was obtained as a light-brown oil (7 mg); λ_{max}(MeOH) nm (log ε) 227 sh (4.08), 278 (3.68), 284 sh (3.63) ν_{max}^{Nujol} cm^{-1} 2835 (NMe), 1618 (OMe), 1612 (Ar C=C), 1028 (OMe); *m/z* 315 (M^+, 40), 300 (11), 151 (90), 136 (28), 121 (100), 106 (24). (Found: C, 72.02; H, 8.08; N, 4.46. $C_{19}H_{25}NO_3$ requires C, 72.3; H, 8.0; N, 4.4). The physical and spectral properties observed for this compound were consistent with those reported for belladine in the lit. [6, 7].

Synthesis of isocraugsodine. A mixture of vanillin (1.52 g) and tyramine (1.38 g) in dry MeOH (50 ml), was stirred at room temp. for 4 hr, under N_2, when isocraugsodine pptd from the soln as orange-yellow crystals (1.64 g), mp and mixed mp of the synthetic sample (with the naturally occurring alkaloid) 218–220° (co-HPLC, MS). A further crop of isocraugsodine (0.98 g) was obtained after concentrating and cooling the methanolic mother liquor.

Transformation of isocraugsodine into (**3**). A soln of isocraugsodine (0.53 g), in MeOH (50 ml), was refluxed for 1 hr. The MeOH soln on concn and cooling pptd isocraugsodine as orange-yellow crystals (0.48 g). The MeOH mother liquor was subjected to prep. TLC using $CHCl_3$–MeOH (9:1) as developer.

12-*Methoxy-9-O-methylnorbelladine* (**3**). Work-up of the prep. TLC layer at R_f 0.65 afforded **3** as an amorphous solid (15.5 mg); λ_{max}(MeOH) 224 (4.31), 282 (3.84), 305 sh (3.66); λ_{max} (MeOH–NaOMe) 232 sh, 284–288, 318 nm; δ_H (CD_3OD) 7.22 (1H, *s*, H-8), 7.01 (2H, *d*, J = 8.5 Hz, H-1, −5a), 6.73 (2H, *d*, J = 8.5 Hz, H-2, −4), 6.40 (1H, *s*, H-11), 3.84 (6H, OMe), 3.61 (2H, *s*, 7-CH_2), 2.78 (4H, *m*, 5- and 6-CH_2); *m/z* 303 (M^+, 41), 302 (17), 301 (22), 196 (18), 181 (11), 167 (100) ($C_9H_{11}O_3$ by accurate mass measurements, 167.070).

Acknowledgements—We are indebted to Dr J. N. Shoolery, (NMR Applications Laboratory, Varian, U.S.A.) and the Analytical Division, Central Drug Research Istitute, Lucknow, India, for some of the spectroscopic data. A.S. and S.K.S. thank the University Grants Commission, New Delhi, for research fellowships.

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Phytochemistry, Vol. 27, No. 6, pp. 1852–1853, 1988.
Printed in Great Britain.

0031-9422/88 $3.00 + 0.00
Pergamon Press plc.

A CYANOGENIC GLUCOSIDE FROM *ILEX AQUIFOLIUM*

MICHAEL WILLEMS

Institut für Pharmazeutische Biologie der Johann Wolfgang Goethe-Universität, Georg-Voigt-Straße 16, D-6000 Frankfurt/Main, F.R.G.

(*Received* 3 *August* 1987)

Key Word Index—*Ilex aquifolium*; Aquifoliaceae; cyanogenic glucoside.

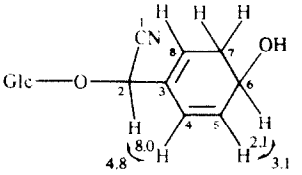
Abstract—A novel cyanogenic glucoside (2-β-D-glucopyranosyloxy-*p*-hydroxy-6,7-dihydromandelonitrile) has been isolated from the ethanolic extract of ripe fruits of *Ilex aquifolium*. Its structure has been established, primarily on the basis of IR, NMR and mass spectral data and its corresponding acetate.

INTRODUCTION

Well-known as a poisonous plant, *Ilex aquifolium* (Holly) is often found in parks and ornamental gardens [1]. Children are regularly poisoned following ingestion of the fruits [2, 3]. Although many compounds have been isolated, the toxin has not yet been identified [1]. The present investigation led to the isolation and identification of a novel cyanogenic glucoside (**1**), occurring in ripe fruits, leaves and stems.

RESULTS AND DISCUSSION

In general, the highest concentrations of cyanogenic glycosides in plants are found in leaves [4]. In this case leaves and stems showed lesser amounts, so **1** was isolated from ripe fruits. Separation of an EtOH extract by column chromatography on silica gel and purification by low pressure column chromatography on RP-18 yielded colourless crystals with a mp of 166–168° (uncorr.). The presence of glucose was established by enzymatic (β-glucosidase) and acidic hydrolysis and TLC. The FABMS spectrum shows a pseudomolecular ion peak at m/z 314 $[M+H]^+$ and after addition of LiJ at m/z 320 $[M+Li]^+$ indicating the M_r to be 313, corresponding to the molecular formula $C_{14}H_{19}NO_7$. **1** readily forms a penta-acetate showing a molecular ion peak in the mass spectrum at m/z 523. The UV spectrum showed λ_{max} (MeOH) at 259 nm. It did not shift on addition of alkali. First indications of the presence of a nitrile was given in the IR spectrum by the characteristic peak at 2212 cm^{-1}, which is in agreement with the signal at δ117.65 in the ^{13}C NMR spectrum [5, 6]. Other important absorptions are at 3200–3600 cm^{-1} (*s*, hydroxyl), 2900–3000 cm^{-1} (*m*, C-H stretching), 1630 cm^{-1} (*m*, conjug. olefins) and at 1010–1190 cm^{-1} (*s*, ether stretching). The ^1H NMR (DMSO) shows a AB-System of two olefinic protons at δ6.23 and 6.14 with a coupling constant of $J = 10$ Hz according to H-4 and H-5. The signal of H-5 is split slightly into a double doublet caused by coupling with the vicinal proton H-6 at δ4.2, $J = 2.3$ Hz (Dieder angle *ca* 90°). Irradiation of the broad doublet of H-6 simplified the double doublet of H-5 to a doublet, collapsed the doublet of the geminal hydroxy group at δ5.15 ($J = 6.6$ Hz) to a singlet and simplified the multiplets at δ2.26 and 1.65 according to the vicinal methylene group H-7a and H-7b ($J = 11.4$ Hz). D_2O exchange caused, a



1

NOEs in per cent.