Tetrahedron. Vol. 24, pp. 1119 to 1131. Pergamon Press 1968. Printed in Great Britain

NARCICLASINE AND NARCIPRIMINE*

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(*Received in the UK* 8 *May* 1967; *accepted for publication* 22 *June* 1967)

Abstract—From fresh bulbs of many varieties of daffodils two compounds have been extracted. Although their structures are related to many Amaryllidaceae alkaloids, the compounds show no basic properties since the nitrogen is amidic in character. The first substance, named *narciclasine*, shows a strong antimitotic activity and has been assigned structure VIII or its mirror image. The second compound, named *narciprimine*, has no antimitotic activity and has been given structure XII.

RECENTLY a new substance, *narciclasine*,[1] with marked antimitotic properties was isolated from the bulbs of several species of daffodils. We now report the structure of narciclasine and of a closely related substance, *narciprimine*, also found in daffodil bulbs but in very small quantities and devoid of any antimitotic activity.

Narciclasine is a crystalline compound, with a strong yellow–green fluorescence, difficult to purify from the usual solvents and as the elimination of the solvents is not easy even under vacuum, analyses always gave unsatisfactory results. Mass spectrum and chemical study point to the formula $C_{14}H_{13}O_7N$. The compound has no basic properties since it is insoluble in dilute mineral acids but it is easily soluble in NaOH and gives with $FeCl_3$ a deep red–violet colour.

The mass spectrum shows the peak of molecular ion at 307 *m/e* and two very strong peaks at 289 (M-18) and 271 (M-36). The IR spectrum indicates the occurrence of a carbonyl group, hydroxy groups and a methylenedioxy group. The UV spectrum in ethanolic solution shows strong absorption maxima, whose wavelengths change in alkaline solution. These facts indicate the presence of phenolic hydroxy groups. The NMR spectrum of narciclasine (100 Mc/s, DMSO-d6, Fig. 1) shows a chelated phenolic OH (S, 13·23 δ), three alcoholic OH groups in a broad absorption (4·4–5·5 δ), an amidic NH group (S, 7·85 δ; it does not exchange with CF_3COOH), a complex absorption of 4 protons between 3·7–4·2 δ, an aromatic proton (S, 6·87 δ), a broad multiplet of one hydrogen at 6·18 δ which does not show any appreciable interaction with the NH (addition of D_2O), and a large singlet of two protons (6·12 δ) due to a methylenedioxy group. This last assignment was possible because in O-methyl-

* Part of this paper has been communicated at the 4th *IUPAC Symposium on the Chemistry of Natural Products*, Stockholm, June (1966).

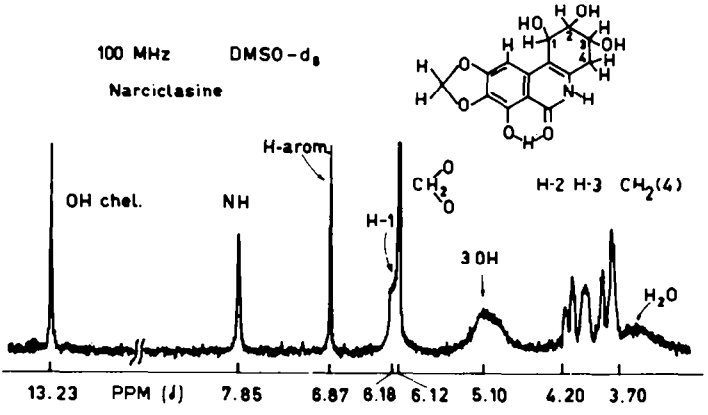


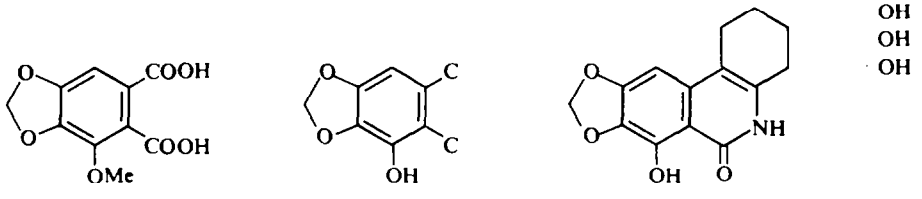
FIG. 1 NMR Spectrum of narciclasine

triacetyl-narciclasine (see later) the same signal is an AB quartet with a geminal coupling of 1·0 c/s characteristic of such a group on a 5-membered ring.

The occurrence of a methylenedioxy group is also confirmed by the evolution of formaldehyde when narciclasine is subjected to sulphuric acid treatment according to the Hansen procedure.[2]

On catalytic hydrogenation narciclasine takes up one mole of hydrogen, giving *dihydronarciclasine* $C_{14}H_{15}O_7N$, with an IR spectrum similar to that of narciclasine, while its UV spectrum presents marked differences, clearly indicating that the hydrogenated double bond is conjugated with the remaining chromophore. Zinc dust distillation of narciclasine yields *phenanthridine*. When treated with an ethanolic solution of CH_2N_2, narciclasine slowly gives O-*methylnarciclasine* $C_{15}H_{15}O_7N$, which is no longer soluble in NaOH and gives no reaction with $FeCl_3$.

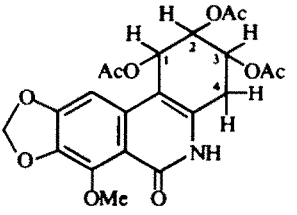
As permanganate oxidation of O-methylnarciclasine in neutral solution yields *cotarnic acid* (I) the presence of fragment II in narciclasine is confirmed. This together with the chelation of a phenolic OH, the occurrence of phenanthridine in zinc dust distillation, the presence of a conjugated double bond and the amidic character of the nitrogen suggest the formula III:



I II III

Treatment of O-methylnarciclasine with Ac_2O and pyridine yields a *triacetyl derivative* which on mild hydrolysis with $Ba(OH)_2$ regenerates O-methylnarciclasine. The structure IV can be assigned to the triacetyl derivative, according to the interpretation of its NMR spectrum.

This clearly shows (60 Mc/s, $CDCl_3$) three acetyl groups (S, 2·08, 2·12, 2·15 δ), a OMe group (S, 4·06 δ), a methylenedioxy group (Q, 6·04 δ, J_{gem} = 1·0 c/s), an aromatic proton (S, 6·80 δ), an amidic NH (7·58 δ) and 5 protons all interacting with



IV

each other. The results of the analysis (100 Mc/s, acetone-d6, Fig. 2) show that these 5 hydrogens are on the same ring. The signals have been attributed to the geminal hydrogens H-4a (4·58 δ) and H-4b (5·18 δ) and to the acetyl-neighbouring protons H-1 (6·27 δ), H-2 (5·40 δ) and H-3 (5·48 δ). The small values of coupling constants, in spite of neighbourhood of the shifts, allow an approximate 1° order analysis, which has been confirmed by double irradiation experiments.

By adding D_2O, the couplings of H-1 and H-4a with NH are removed: the small value of couplings ($J < 0{\cdot}5$ c/s) are detectable only by the sharpening of the 6·27 and 4·58 δ signals; the other protons are unchanged.

H-1 couples with H-2 ($J_{1,2} = 4{\cdot}5$ c/s), H-3 ($J_{1,3} = 1{\cdot}0$ c/s) and H-4a ($J_{1,4a} = 2{\cdot}2$ c/s) giving an eight line pattern. Upon irradiation of H-2 ($\Delta\nu = +88$ c/s) $J_{1,2}$ vanishes and H-1 becomes a quartet with splitting of 1·0 and 2·2 c/s (Fig. 2a). Irradiation of H-3 ($\Delta\nu = +84$ c/s) also perturbs H-2 according to the small shift difference (Fig. 2b), while, irradiation of H-1 decouples H-2 with $\Delta\nu = -86$ c/s and H-3 with $\Delta\nu = -82$ c/s (Fig. 2d): H-3 changes from a triplet of doublets ($J_{2,3} = \sim J_{3,4a} = 2{\cdot}3$–$2{\cdot}4$ c/s; $J_{1,3} = 1{\cdot}0$ c/s) into a triplet; the eight line pattern of H-2 ($J_{2,3} = 2{\cdot}4$ c/s; $J_{1,2} = 4{\cdot}5$ c/s; $J_{2,4a} = 1{\cdot}2$ c/s) collapses into a slightly broadened doublet, with $J_{2,4a}$ not completely resolved.

The last coupling of H-1 ($J_{1,4a} = 2{\cdot}2$ c/s) is removed by irradiation of H-4*a* ($\Delta\nu = +170$ c/s): it leads to a double doublet with splitting of 4·5 and 1·0 c/s (Fig. 2c). Reversal experiment ($\Delta\nu = -174$ c/s) converts the H-4a signal into two doublets of 9·0 and 1·2 c/s ($J_{4a,4b}$ and $J_{2,4a}$ resp.) (Fig. 2g).

The strong coupling of 9·0 c/s (geminal interaction of the protons at C-4) vanishes upon irradiation of H-4a ($\Delta\nu = +58$ c/s): the two doublets of H-4b collapse in one doublet of 2·3 c/s ($J_{3,4b}$); the reverse experiment ($\Delta\nu = -58$ c/s) leads H-4a to a broad signal, the line width of which measures 4·7 c/s, being consistent with 1,4a and 2,4a couplings (Figs. 2f and 2h).

The last coupling ($J_{2,4a} = 1{\cdot}2$ c/s) is proved by irradiation of H-4a ($\Delta\nu = +87$ c/s): the H-2 signal goes into a doublet of doublets with splitting of 4·5 and 2·4 c/s (Fig. 2e).

An open-chain alternative structure can be excluded in accordance with: (a) the number of protons; (b) the existence of an homoallylic coupling between H-4a and H-1; (c) the evidence of interaction between H-4a and NH; (d) the strong shift difference between the geminal protons H-4a and H-4b, according to their different orientation to the amidic CO plane.

The alternative structure with the CH_2 at position 1 can be ruled out as the strong downfield shift of both protons of the methylene cannot be based only on their allylic nature, the acetyl long-range effects or the angular aromatic system. The deshielding is better explained by the near amidic nitrogen, having a strong localized positive

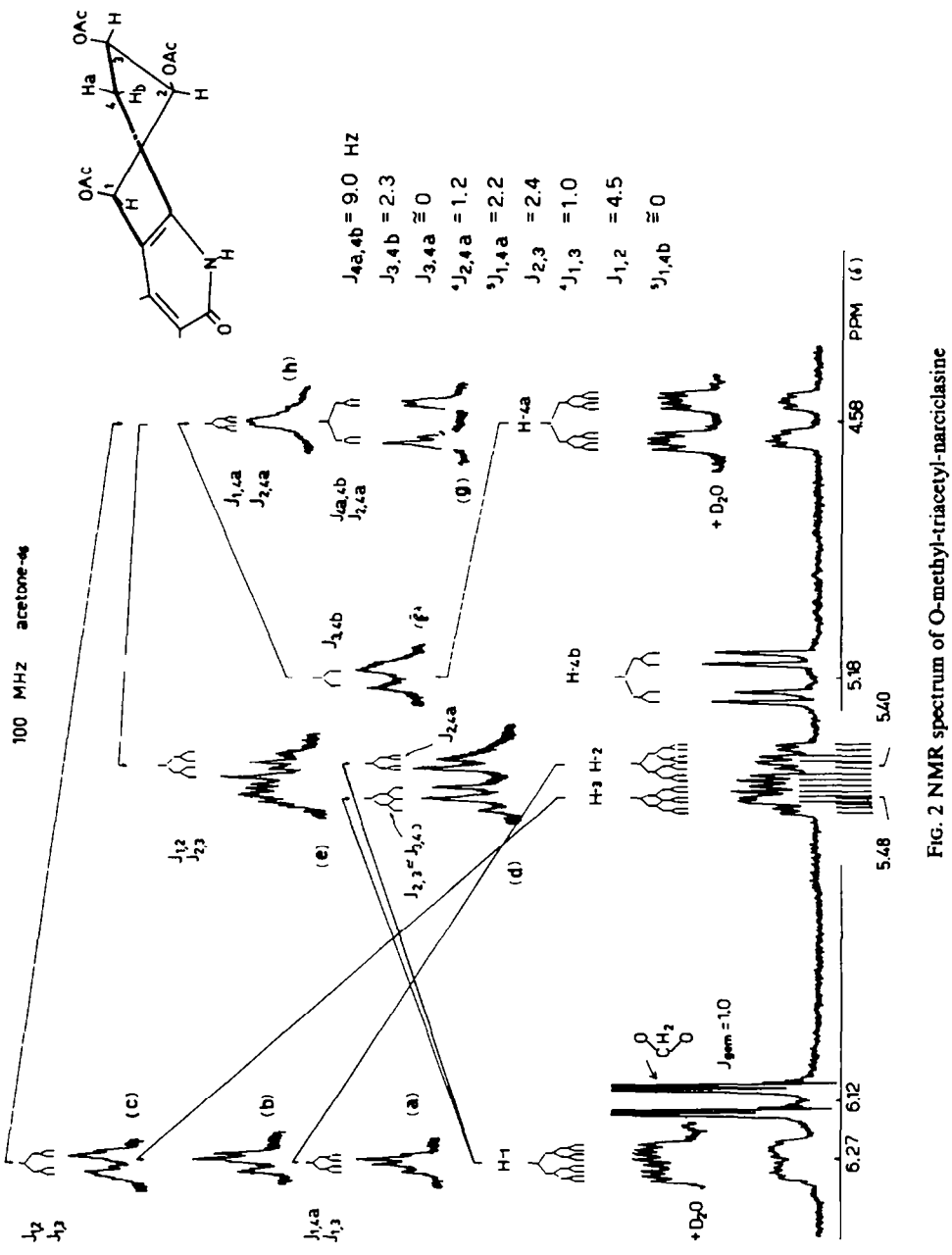


FIG. 2 NMR spectrum of O-methyl-triacetyl-narciclasine

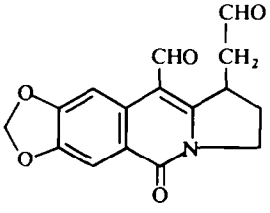
charge (see NMR spectrum of XI). Positions 2 and 3 for the CH_2 are immediately excluded by chemical shifts values.

Position 4 being assigned to CH_2, the sequence of OH groups on the ring is proved and the structure of narciclasine is that shown in Fig. 1.

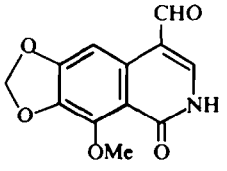
The occurrence of three vicinal OH groups is supported by periodic acid oxidation of O-methylnarciclasine. 2·5–2·8 moles of HIO_4 being required. If after oxidation the solution is heated at 100°, 0·60 moles of formaldehyde are evolved. When the oxidation is performed with only 2 moles of HIO_4, occurrence of 0·60 moles of formic acid may be detected.

Periodic acid oxidation of O-methylnarciclasine yields a substance $C_{12}H_9O_5N$ which gives a positive test with 2,4-dinitrophenylhydrazine and has been named *narciclasic aldehyde*. Its IR spectrum is indicative of the amidic group, an aldehydic CO and a methylenedioxy group. Its UV spectrum closely resembles the one described for the dialdehyde VI obtained by HIO_4 oxidation of dihydrolycorinone.[3] The NMR spectrum presents signals for OMe, CHO, methylenedioxy group, amidic NH, the aromatic proton at 8·20 δ, and a vinyl proton at 7·96 δ (broad doublet, $J = 6$ c/s): the latter collapses to a singlet by adding D_2O.

The strong downfield shift of the aromatic proton, due to the *peri* aldehydic group, and the coupling of the vinylic proton with NH rule out the possibility of placing the aldehyde group on C-3 of the isocarbostyril system. From these results, narciclasic aldehyde can be expressed by structure VII, which is also supported by zinc dust distillation, yielding isoquinoline:



VI



VII

The isolation of an isocarbostyril derivative with position 3 free cannot be easily explained. However, the evolution of formaldehyde in addition to formic acid suggests a mechanism based either on cleavage or on further oxidation of not isolated intermediates coming from an isocarbostyril derivative with a CH_2—CHO chain at position 3: the latter is the logical product of the initial cleavage of the bonds between C-1 and C-2 resp. C-2 and C-3 by 2 moles of HIO_4, and is vinylogous of malonic dialdehyde.*

Some information on the relative configuration of the three OH groups may be obtained from the values of coupling constants in the NMR spectrum of IV.

No coupling is observed between the vicinal protons H-3 and H-4a: thus, when considering the Karplus relation[5] to be valid also without knowing the K value to be used for these frameworks, the dihedral angle $\vartheta_{3,4a}$ is required to measure 80–110°: which is easily obtained with a slightly twisted chair conformation (C-3 downwards, as in Fig. 2) and with the OAc group at C-3 in axial position.

The OAc group at C-1 should be axial, because the homoallylic couplings are almost

* Easy cleavage of malonic dialdehyde into three moles of HCOOH by HIO_4 oxidation has been reported.[4]

null ($J_{1,4b} = \sim 0$) and remarkably high ($J_{1,4a} = 2{\cdot}2$ c/s). To satisfy these conditions H-1 and H-4b must lie in the double bond plane or form small angles (20–40°) with it, while H-4a must form an angle of about 70–100°* with the same plane. These requirements are fulfilled in the above suggested conformation, with the OAc group on C-1 in axial position.

The H-1 is remarkably deshielded. This is in agreement with an equatorial orientation, since it lies almost on the same plane as the aromatic system and is very near to it.†

The other angles agree with the J values, taking into account that the substituent effects (amidic nitrogen included, with its localized positive charge) cannot be disregarded.

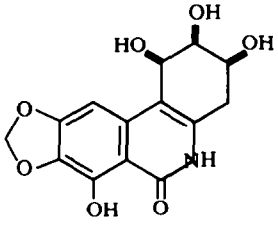
Two other small coupling constants are measured: $J_{1,3} = 1{\cdot}0$ c/s and $J_{2,4a} = 1{\cdot}2$ c/s. H-1 and H-3 are disposed in a planar W configuration; for H-2 and H-4a, no W arrangement is possible for any orientation of H-2. The stereospecificity of these constants across 4σ bonds is known:[8] a planar zig-zag (W) geometry has been postulated as a condition of maximum coupling, but many examples are known of couplings also in a non-W arrangement:‡ the substituent effects here too may be important.

The OAc group at C-2 is probably equatorial as otherwise the molecule would exist in the more stable chair conformation, which would not be in agreement with the observed coupling values.

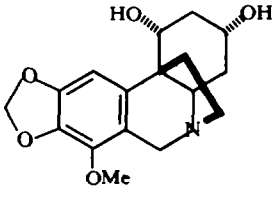
The chair conformation with equatorial OAc at C-1 and C-3 and axial OAc at C-2 must be excluded, as otherwise the interpretation of the two homoallylic couplings between H-1 with H-4a and H-4b would be impossible and as it would not agree with the shift of H-1, which should be almost perpendicular to the plane of the aromatic system. Moreover, $J_{3,4a} = \sim 0$ c/s could be otherwise justified only by a very strong twisting of the ring.

Boat conformations and the two quickly interconvertible chair conformations may be excluded as they do not agree with the couplings.

Therefore, the following structure VIII or its mirror image is proposed for narciclasine:



VIII

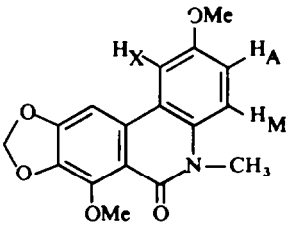


IX

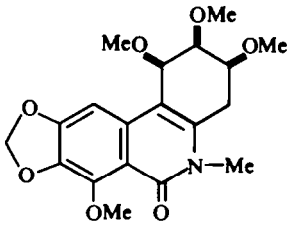
* See the case of eleutherine and isoeleutherine.[6]

† Compare the strong deshielding ($\Delta \sim 1$ ppm) of angular protons (H-4 and H-5) in phenanthrene ($\delta_3 = 7{\cdot}88$, $\delta_4 = \delta_5 = 8{\cdot}93$; $CDCl_3$).[7]

‡ See also the abnormal couplings quoted in the Barfield paper,[8] footnotes 12, 13, 14 and Ref. 9.



X



XI

A conformation of ring C with axial substituents at C-1 and C-3 is not unusual, being presented also by *nerbowdine* (IX).[10] Careful investigation of both Dreiding and Stuart models shows that an axial OAc at C-1 is less hindered than an equatorial OAc.*

Further support for the structure of narciclasine was obtained by Kuhn methylation experiments. Narciclasine yields two products: crystalline *permethyl-iso-narciprimine* $C_{17}H_{15}O_5N$ (X) and an oily *permethyl-narciclasine* $C_{19}H_{23}O_7N$ (XI). The structures of these compounds are proved by their NMR spectra.

The NMR spectrum (100 Mc/s, acetone-d6) of X shows a N—Me (S, 3·67 δ), two OMe (S, 3·93 and 3·98 δ), a methylenedioxy group (S, 6·18 δ), an aromatic proton on ring A (S, 7·60 δ) and three aromatic protons in an AMX pattern coupled with *ortho*, *meta* and *para* couplings. H_X is a slightly broadened doublet at 7·67 δ (J_{AX} = 2·5 c/s, J_{MX} = ~0·5 c/s), H_A a double doublet at 7·14 δ (J_{AX} = 2·5 c/s, J_{AM} = 8·5 c/s), H_M a slightly broadened doublet at 7·36 δ (J_{AM} = 8·5 c/s, J_{MX} = ~0·5 c/s).

The NMR spectrum (60 Mc/s, $CDCl_3$) of XI shows the N—Me (S, 3·24 δ), one aromatic OMe (S, 4·05 δ), three aliphatic OMe (S, 3·47, 3·52, 3·58 δ), a methylenedioxy group (Q, J = ~1 c/s, 6·00 δ) and one aromatic proton (S, 6·68 δ). The other hydrogens on ring C give a complex pattern between 3·5 and 4·3 δ (4H) partially overlapped by Me absorptions, while H-1 (broad D at 6·13 δ) is still strongly deshielded by the aromatic system in front of it,† (see NMR spectrum of IV). The two protons at C-4 are shifted upfield (~4 δ) to IV because of the electron-releasing Me on the amidic nitrogen, and the loss of the acetyls long-range effect.

The different aromatization of narciclasine (VIII) to narciprimine (XII) (see later) by acids or to a derivative X of isonarciprimine by Ag_2O + MeI supports the relative stereochemistry suggested for VIII.

In the first case the probable pathway is: protonation of the equatorial OH at C-2 (easier than protonation of the axial OH at C-1), E_1 loss of water and formation of the carbocation at C-2 (probably more stable than the isomeric carbocation at C-1, because of the hydrogen bond between the OH at C-1 and C-3), release of axial H-1 as H^+, further elimination of water from axial OH at C-3 and one of the hydrogens at C-4. The relative stability of the carbocations and their configuration (tetrahedral or planar) is a matter of speculation.

* Substituents on positions 4 and 5 of a tricyclic angular system (e.g. phenanthrene) can be subjected to out-of-plane distortions by molecular overcrowding: for derivatives of 4,5-dimethylphenanthrene.[11] The same molecular overcrowding was observed on 3,4-benzophenanthrene.[11a] As in structure VIII the equatorial substituent on C-1 is almost coplanar with rings A and B, the conformation with the bulkier substituent in axial condition seems to be the less hindered one.

† See second footnote on p. 1124.

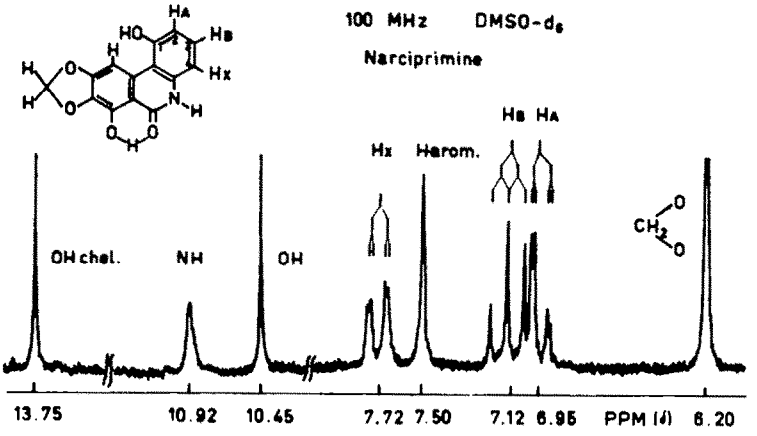


FIG. 3 NMR spectrum of narciprimine, XII

In the second case the rates of O-methylation and of elimination can be competitive: the equatorial OH at C-2 undergoes faster methylation than axial OH at C-1 and C-3, the latter being subjected to faster elimination according to the normal *trans*-diaxial E_2 mechanism.

Narciprimine

The other constituent of daffodil bulbs, is a crystalline substance with no basic properties and a positive $FeCl_3$ test. Analyses were unsatisfactory, but the analysis of a derivative, chemical degradation and NMR evidence points to the formula $C_{14}H_9O_5N$. Its IR spectrum closely resembles that of narciclasine, showing the characteristic pattern for a methylenedioxy group, a lactam and OH groups. The UV spectrum of narciprimine is quite different from that of narciclasine.

The NMR spectrum of narciprimine (100 Mc/s, DMSO-d6, Fig. 3) accounts for 9 protons: a methylenedioxy group (S, 6·20 δ), an aromatic proton (S, 7·50 δ), an amidic NH (S, 10·92 δ), one chelated OH (S, 13·75 δ), another phenolic OH (S, 10·45 δ), and three aromatic protons strongly coupled with each other in an ABX pattern: H_X is a double doublet at 7·72 δ (J_{AX} = 1·5 c/s, *meta* coupling; J_{BX} = 7·8 c/s, *ortho* coupling), H_B a triplet at 7·12 δ ($J_{AB} = J_{BX}$ = 7·8 c/s, *ortho* couplings), H_A a double doublet at 6·95 δ (J_{AB} = 7·8 c/s, *ortho* coupling; J_{AX} = 1·5 c/s, *meta* coupling). The two *ortho* couplings of H_B indicate the sequence of three vicinal protons.

The substance does not take up hydrogen in the presence of catalysts; it gives *di-O-methylnarciprimine* on treatment with ethanolic diazomethane, and *diacetylnarciprimine* with Ac_2O and pyridine. On oxidation with neutral permanganate, di-O-methylnarciprimine yields *cotarnic acid* and zinc dust distillation of narciprimine yields *phenanthridine*.

In accordance with these results, structure XII in Fig. 3 has been assigned to narciprimine. The alternate structure with the OH group at C-4 cannot be excluded by the NMR spectrum of narciprimine alone,* but it can be ruled out as narciclasine itself changes into narciprimine by loss of two moles of water on treatment with cold hydrochloric acid.

* In this case, also H-1 should be strongly deshielded, see Ref. 7 and second footnote p. 1118.

As the structures of narciclasine and narciprimine are clearly related to many Amaryllidaceae alkaloids, the occurrence of narciclasine in many species of *Narcissus* is undoubtedly connected with the biosynthesis of these alkaloids.

EXPERIMENTAL*

Extraction and purification. Fresh daffodil bulbs (1 Kg) were ground in 95% EtOH (1 l.) and left for 24 hr. After filtration on Büchner funnel through cotton gauze, the residue was carefully squeezed and washed with EtOH; the aqueous–ethanolic soln was concentrated under reduced press until the EtOH had been eliminated (residual volume, about 300 ml). The soln was heated to 80°, 15 g of Celite 545 added and filtered hot, and the filter washed with a little water. The clear yellowish soln had a pH 5 and a strong yellow-green fluorescence. It was extracted 5 times with 750 ml (total) of BuOH, and the butanolic extract evaporated to dryness under reduced press. The residue (about 15 g) was kneaded with a little EtOH and 20 g of silica gel (Merck, under 0·08 mm), carefully dried under vacuum and charged by AcOEt on a chromatographic column (dia 50 mm, wt of silica gel 60 g). Elution with AcOEt, the first 300 ml were discarded; the following 300 ml contained small quantities of *narciprimine* mixed with several impurities. When no more product was eluted, elution with AcOEt–MeOH 95:5 (about 1 l.) yielded after evaporation under reduced press. a whitish residue which crystallized from 25 ml AcOH, giving from 60 to 150 mg of *narciclasine* (depending on the varieties of daffodils). Narciclasine was further purified from AcOH. On TLC (silica gel G Merck, eluent AcOEt 10, EtOH 2, H_2O 1) the two substances exhibit the same strong yellow-green fluorescence with $R_f = 0{\cdot}75$ (narciprimine) and $R_f = 0{\cdot}55$ (narciclasine).

The AcOEt fractions of many chromatographic separations were collected, evaporated to an oily consistency and treated with hot AcOH, from which *narciprimine* crystallized in needles.

While from a kg of bulbs 60–150 mg of narciclasine was obtained, the same weight yielded only 3–5 mg of narciprimine. When extracting some varieties of daffodils (e.g. "Helios") particularly rich of narciclasine (up to 200 mg/kg), a simplified procedure could be used. The clear yellowish soln after Celite treatment was treated with 30 g NaCl, extracted 3 times with $CHCl_3$ (discarded), then extracted 5 times with 500 ml AcOEt (total). The AcOEt extract was washed with sat. NaCl aq, dried and evaporated. The residue was taken up in 20 ml boiling AcOH, from which crude narciclasine crystallized. The mother liquor contained narciprimine and some narciclasine.

The present research has been accomplished by working on a total of 2·0 g narciclasine, mainly extracted from the following sources: *Narcissus tazetta* L., wild in western Sicily; *N. incomparabilis* Mill., var. "Helios" and "Sempre Avanti"; *N. pseudonarcissus* L., var. "King Alfred"; *N. triandrus* L., var. "Thalia" and "Tresamble".

Narciclasine

Soft, lightly yellowish needles, with strong yellow-green fluorescence, m.p. 232–234° dec. (from AcOH or from methylcellosolve-water). (Found:† C, 53·41; H, 4·44; N, 4·55. $C_{14}H_{13}O_7N$ requires: C, 54·72; H, 4·26; N, 4·56%.) Optical activity ($c = 1{\cdot}5$, EtOH): $[\alpha]_{589} = +145°$, $[\alpha]_{546} = +198°$, $[\alpha]_{436} = +538°$, $[\alpha]_{364} = +983°$.‡

IR spectrum (KBr): 3280 cm^{-1} (broad, OH); 1675 cm^{-1} (NH–CO); 1376, 1274, 1232, 1088, 1031, 925 cm^{-1} (methylenedioxy group) §.

*IR and UV spectra were recorded on Perkin–Elmer Infracord 137 and Beckman DK-2 spectrophotometers. NMR spectra at 60 Mc/s were run on a Varian A-60 spectrometer; the integrals were measured with a Hewlett–Packard CR-405 Digital Voltmeter. For NMR spectra at 100 Mc/s a Varian HR-100 spectrometer was used: we wish to thank Dr. Priv. Doz. W. von Philipsborn for having kindly allowed one of us (R.M.) to use the spectrometer in the Organic Chemistry Institute of the University of Zürich. Chemical shifts are expressed in ppm (δ units), with TMS as internal standard. Double irradiation experiments were performed in "field sweep"; the differences $\Delta\gamma$ between the two modulation frequencies are taken to be positive when the irradiated signal falls at higher fields in respect to the observed signal, and *vice-versa*.

† Unsatisfactory analyses were always obtained for narciclasine and narciprimine, owing to the presence of solvent.

‡ Recorded on a Perkin–Elmer 141 photoelectric polarimeter.

§ Band assignment of methylenedioxy group.

UV spectrum (EtOH): λ_{max} 329, 302, 252 nm, log ε = 3·52, 3·62, 4·25; (0·01 N NaOH): λ_{max} 355, 310, 249, 219 nm, log ε = 3·57, 3·46, 4·18, 4·11.

NMR spectrum (100 Mc/s DMSO-d6), see introduction.

Mass spectrum:* 307 m/e (M^+), 289 (M-18), 271 (M-36).

Colour tests. Dragendorff: negative. $FeCl_3$: deep red-violet colouring.

TLC: R_f = 0·55 (eluent AcOEt 10, EtOH 2, H_2O 1); R_f = 0·65 (eluent AcOEt 10, EtOH 3, H_2O 1).

Hansen reaction for methylenedioxy group. 3 mg narciclasine were dissolved in 0·5 ml 88% H_2SO_4 and heated at 60°: a drop of 1% solution of chromotropic acid in 72% H_2SO_4 suspended over the soln showed the characteristic violet colouring after some min.

Dihydronarciclasine. Narciclasine (92 mg, 0·3 mmoles) was dissolved in 10 ml EtOH and hydrogenated on 10% Pd–C at room press and temp: 6·6 ml H_2 (at 0° 760 mmHg) were absorbed (= 0·98 double bonds). After filtration and evaporation of the EtOH under reduced press, the product crystallized from benzene–EtOH, m.p. 168–170°. (Found: C, 54·34; H, 5·28. $C_{14}H_{15}O_7N$ requires: C, 54·37; H, 4·89%.) On TLC (eluent AcOEt 10, EtOH 3, H_2O 1) two very near spots were observed, R_f = 0·63 and 0·58 resp. (the second one seemed to be slightly more abundant): the product was a mixture of two diastereoisomers.†

IR spectrum (Nujol mull): 3300 cm^{-1} (OH and NH), 1670 cm^{-1} (CO–NH), 1335, 1280, 1224, 1080, 1032, 935 cm^{-1} (methylenedioxy group). UV spectrum (EtOH): λ_{max} 309 (sh.), 279, 235, 212 nm, log ε = 3·36, 3·85, 4·32, 4·20. The substance has a very weak blue fluorescence and gives positive test with $FeCl_3$.

Zinc dust distillation of narciclasine phenanthridine

Narciclasine (100 mg) was mixed in a test tube with 2 g of a mixture of 3 parts Zn dust, 1 part $ZnCl_2$ and 1 part NaCl (previously melted and finely ground). The mixture was carefully heated by direct flame: a yellowish product sublimed and was collected in Et_2O and resublimed: m.p. 102°, no depression when mixed with an authentic sample of *phenanthridine*‡ having the same m.p. On TLC (eluent n-hexane 2, AcOEt 1) both products showed a strong blue fluoroescent spot, R_f = 0·35. On GLC (Wilkens Aerograph Hy-Fi A-600 with flame ionization detector, 10 fr × ¼ in. column packed with 5% SE-30 methylsilicone on acid-washed Chromosorb W 60–80 mesh, temp. 225°, carrier gas N_2 1·2 atm) both products presented a single peak after 9 min. (also on crossed injection). UV and IR spectra were superimposable. Phenanthridine was also obtained when narciclasine was heated with Zn dust alone.

O-methylnarciclasine. Narciclasine (100 mg) was dissolved in 50 ml EtOH and treated with a large excess of an ethanolic soln of CH_2N_2; after 48 hr, the soln was evaporated to dryness under reduced press and the residue crystallized from AcOH or from EtOH–benzene giving shiny needles with a very strong blue fluorescence, m.p. 206°. (Found: C, 56·12; H, 4·91; N, 4·29. $C_{15}H_{15}O_7N$ requires: C, 56·07; H, 4·71; N, 4·36%.) IR spectrum (KBr): 3350 cm^{-1} (OH and —NH—), 1655 cm^{-1} (—CO—NH), 1380, 1230, 1090, 1030, 930 cm^{-1} (methylenedioxy group). UV spectrum (EtOH): λ_{max} 298, 288 nm, log ε = 3·68, 4·40. The substance was insoluble in NaOH and gave a negative reaction with $FeCl_3$. TLC: R_f = 0·50 (eluent: AcOEt 10, EtOH 2, H_2O 1).

O-methyl-triacetyl-narciclasine. O-methylnarciclasine (100 mg) was dissolved with 1 ml Ac_2O and 1 ml pyridine: after 48 hr at room temp, the soln was carefully evaporated to dryness under reduced press at a temp not exceeding 20°. The oily residue was purified by preparative TLC (silica gel G Merck, thickness 2 mm, eluent AcOEt 4, n-hexane 1) collecting the band with R_f = 0·50 having a strong blue fluorescence. The product was amorphous, very soluble in every solvent except n-hexane and could not be crystallized. IR spectrum (KBr): 3300–3200 cm^{-1} (—NH—CO—), 1745 cm^{-1} (—O—COMe), 1660 cm^{-1} (—CO—NH—), 1370, 1080, 1030, 930 cm^{-1} (methylenedioxy group), 1230 cm^{-1} (—O—COMe). UV spectrum (EtOH): 305, 250 nm, log ε = 3·82, 4·58. NMR spectrum (60 Mc/s, $CDCl_3$; 100 Mc/s, acetone-d6): see Introduction. The soln of O-methyltriacetyl-narciclasine (50 mg) in 15 ml MeOH was added to 5 ml 2N $Ba(OH)_2$ heated on steam bath until the formation of white flakes ceased, then it was added to 20 ml MeOH and saturated with CO_2. After filtering, the soln was evaporated to dryness and the residue crystal-

* The spectrum has been registered by Dr. A. Selva (Istituto di Chimica, Politecnico di Milano) to whom out thanks are due. An Hitachi–Perkin Elmer RMU-6D single focusing spectrometer was used, with direct injection into the ionic source at 140°, ionization potential 70 V, total emission 80 μA.

† This opinion is confirmed by the NMR spectrum of the product (not discussed here), in which many signals are split.

‡ Prepared by Zn dust distillation of phenanthridone. Pictet *et al.*[13] indicate m.p. 104°.

lized from AcOH as needles, m.p. 206°, no depression in a mixed m.p. with O-methylnarciclasine, IR and UV spectra superimposable.

Permanganic oxidation of O-methylnarciclasine—Cotarnic acid

O-methylnarciclasine (30 mg) was dissolved at 40–50° in 100 ml H_2O: about 5 ml of 3% $KMnO_4$ were dropped into the cold soln till persistent coloration (disappearing after 48 hr). The soln was filtered, acidified at Congo red by 50% H_2SO_4 and extracted 3 times with Et_2O; from this a white powder was obtained which was melted and heated at 180°; after cooling, prismatic needles were obtained, m.p. 161–162° also when mixed with *cotarnic anhydride* with the same m.p.* The product was treated 3 times with MeOH and $MeNH_2$, evaporated to dryness each time, heated to 210° for 5 min and sublimed at 120–130° under 1 mm Hg: small crystals were obtained, m.p. 205–206° (change of crystal form at 175–180°), and identical in the appearance and the behaviour with *cotarnic acid N-methylimide*, m.p.* 205°; mixed m.p. no depression: UV and IR spectra were superimposable. UV spectrum (EtOH): 334, 248, 230 nm, log ε = 3·38, 4·38, 4·20. IR spectrum (Nujol mull): 1765, 1715 cm^{-1} (CO—N<); 1245, 1098, 1044, 927 cm^{-1} (methylenedioxy group).

Oxidation of O-methylnarciclasine with HIO_4. Titration with HIO_4 at room temp: after 24 hr, 2·5, 2·7, 2·8 equivs HIO_4 were required in 3 different experiments performed on 0·3 mmoles of O-methylnarciclasine.

When the oxidized soln was heated to b.p., *formaldehyde* was evolved (0·6 moles/mole), and identified as *dinitrophenylhydrazone* m.p. 166° also when mixed with an authentic specimen. In the soln the presence of *narciclasic aldehyde* (see later) was detected.

When O-methylnarciclasine was treated with 2 moles HIO_4, distillation gave 0·6 moles of HCOOH; use of excess HIO_4 was prevented by formation of iodine.

Periodic acid oxidation of O-methylnarciclasine—Narciclasic aldehyde

O-methylnarciclasine (60 mg) was dissolved in 60 ml H_2O, then 40 ml 0·1M HIO_4 was added; after 24 hr, the yellow ppt was collected and crystallized from EtOH as yellowish needles (20 mg; 45%), m.p. 282–283°. (Found: C, 57·96; H, 4·26. $C_{12}H_9O_5N$ requires: C, 58·30; H, 3·67%.) IR spectrum (KBr): 3100 cm^{-1} (NH); 2700 cm^{-1} (aldehyde, C—H stretching); 1680 cm^{-1} (aldehyde, C=O stretching); 1640 cm^{-1} (CO—NH); 1265, 1215, 1101, 1037, 925 cm^{-1} (methylenedioxy group).

UV spectrum (EtOH): 343, 330, 260, 245 nm, log ε = 3·92, 3·95, 4·33, 4·54†.

NMR spectrum (60 Mc/s, DMSO-d6): OMe (S, 3·88 δ), methylenedioxy group (S, 6·13 δ), —NH—CH= (broad D, J = 6 c/s, 7·96 δ), H_{arom} (S, 8·20 δ), CHO (S, 9·50 δ), NH (broad 11·71 δ); when treated with D_2O, the doublet at 7·96 δ changes into a sharp singlet.

Zinc dust distillation of narciclasic aldehyde—Isoquinoline

Narciclasic aldehyde (15 mg) was treated in a test tube with 1 g Zn dust, $ZnCl_2$ and NaCl mixture as previously described for narciclasine: distillation of yellow droplets, having a strong isoquinolinic odour were collected in 2 drops EtOH. A sample, examined by GLC (Wilkens Aerograph Hy-FI A-600 with flame ionization detector, 4 ft. × ⅛ in. column packed with 3% D.E.G.S. on alkali-washed Chromosorb, temp. 137°, carrier gas N_2 1 atm), gave only a unitary peak after 21 min corresponding to the one of *isoquinoline*, also on crossed injection. Another sample gave an UV spectrum superimposable on the one of isoquinoline.

Transformation of narciclasine into narciprimine. Narciclasine (100 mg) was mixed with 5 ml cold conc HCl: the substance slowly changed from soft needles into an heavy powder, which was collected after 12 hr and crystallized from a large volume of EtOH. About 50 mg of pure product were obtained, identical with (TLC, UV, IR and NMR spectra) natural narciprimine.

Narciprimine

Small yellowish needles (from AcOH or from EtOH), dec. without melting at 300–320°. No satisfactory analysis was obtained.

* For the preparation of cotarnic acid anhydride and methylimide, see W. Roser.[14] Our products, prepared starting from cotarnine, closely agree with the data and m.p. reported in literature.

† The dialdehyde from dihydrolycorinone[3] has λ_{max} 346, 332, 309, 266, 249 nm, log ε = 3·97, 3·96, 3·86, 4·36, 4·40.

IR spectrum (KBr): 3450, 3290, 3100 cm^{-1} (OH and NH); 1675 cm^{-1} (CO—NH); 1372, 1282, 1242, 1086, 1033, 933 cm^{-1} (methylenedioxy group). UV spectrum (EtOH): 353, 337, 322, 295, 274, 257, 233 nm, log ε = 3·89, 3·86, 3·67, 3·96, 4·24, 4·72, 4·38. NMR spectrum (100 Mc/s, DMSO-d6): see Introduction. TLC: R_f = 0·75 (eluent AcOEt 10, EtOH 2, H_2O 1). Colour tests. $FeCl_3$: deep red-violet colouring.

Di-O-methylnarciprimine. Narciprimine (50 mg) was dissolved in an EtOH soln of CH_2N_2 and left at room temp for 24 hr; the methylated product was very slightly soluble in EtOH (and in other solvents), and separated as an amorphous, uncrystallizable solid, m.p. > 320°. It had a strong violet fluorescence and gave a negative test with $FeCl_3$. IR spectrum (KBr): 3200 cm^{-1} (NH—CO); 1680 cm^{-1} (CO—NH); 1270 cm^{-1} (aromatic OMe); 1335, 1293, 1245, 1085, 1033, 940 cm^{-1} (methylenedioxy group). UV spectrum (EtOH, qualitative): 355, 339, 298, 273 (sh.), 257, 232 nm.

Diacetylnarciprimine. Narciprimine (80 mg) was treated with Ac_2O (0·5 ml) and pyridine (0·5 ml) during 24 hr at room temp. After usual work-up, the diacetyl derivative was crystallized from MeOH, m.p. 247°, weak green-blue fluorescence. (Found: C, 60·80; H, 3·62. $C_{18}H_{13}O_7N$ requires: C, 60·85; H, 3·68%.) UV spectrum (EtOH): 344, 328, 269, 252, 247 nm, log ε = 3·93, 3·93, 4·20, 4·71, 4·73. IR spectrum (KBr): 3175 cm^{-1} (NH—CO); 1760 cm^{-1} (phenolic ester); 1650 cm^{-1} (CO—NH); 1361, 1292, 1245, 1081, 1030, 934 cm^{-1} (methylenedioxy group) NMR spectrum (60 Mc/s, DMSO-d6): 2 OAc (S, 2·37–2·42 δ), methylenedioxy group (S, 6·28 δ), H_{arom} ring A (S, 8·00 δ), NH (S, 11·17 δ), 3 aromatic protons in an ABX pattern (X part, H-4, 8·17 δ; AB part, H-2 and H-3, 7·1–7·4 δ).

Zinc dust distillation of narciprimine—Phenanthridine

Narciprimine (15 mg) was treated in a test tube with 1 g of the mixture of Zn dust, $ZnCl_2$ and NaCl as previously described for narciclasine. The sublimed product, collected by Et_2O and resublimed, was identical (UV spectrum, TLC and GLC) with the products obtained from narciclasine and from phenanthridone.

Permanganic oxidation of di-O-methylnarciprimine—Cotarnic acid

Di-O-methylnarciprimine (prepared by CH_2N_2 treatment of 30 mg narciprimine) was dissolved in 300 ml acetone and treated with excess $KMnO_4$ on steam bath during 6 hr. After destroying the excess $KMnO_4$ by H_2O_2 and usual work-up, the crude product was transformed into the anhydride and into *cotarnic acid N-methylimide*, m.p. 204–205°; mixed m.p. with an authentic sample showed no depression; UV and IR spectra were superimposable.

Kuhn methylation of narciclasine—Permethyl-isonarciprimine and permethylnarciclasine

Narciclasine (300 mg) was treated with 25 ml dimethylformamide, 15 ml MeI and 10 g Ag_2O. The crude product was chromatographed on silica gel (Merck, 0·05–0·20 mm), eluting with benzene and then with benzene–AcOEt (3:1). From the benzene fractions *permethyl-isonarciprimine* was obtained, shiny white needles with a blue fluorescence, m.p. 175–176° from MeOH. (Found: C, 65·62; H, 5·01. $C_{17}H_{15}O_5N$ requires: C, 65·17; H, 4·82%); IR spectrum (KBr): 1640 cm^{-1} (CO—N<); 1395, 1280, 1224, 1089, 1035, 931 cm^{-1} (methylenedioxy group). UV spectrum (EtOH): 362, 348, 318, 304, 276, 252, 229 nm, log ε = 3·91, 3·98, 3·68, 3·82, 4·03, 4·63, 4·43. NMR spectrum (100 Mc/s, acetone-d6): see Introduction. TLC: R_f = 0·60 (eluent AcOEt).

From the benzene–AcOEt fractions *permethyl-narciclasine* was obtained as a thick oil with a very strong violet fluorescence. IR spectrum (liquid): 1650 cm^{-1} (CO—N); 1245, 1084, 1036, 932 cm^{-1} (methylenedioxy group). UV spectrum (EtOH): 302, 249 nm, log ε = 3·75, 4·51. NMR spectrum (60 Mc/s, $CDCl_3$): see Introduction. TLC: R_f = 0·33 (eluent AcOEt).

Acknowledgement—We gratefully thank the Consiglio Nazionale delle Ricerche for financial support.

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