Tetrahedron. Vol. 30, pp. 2233 to 2237. Pergamon Press 1974. Printed in Great Britain

CRITERIA FOR THE DETERMINATION OF THE STEREOCHEMISTRY OF STEROIDAL INDOLIZIDINES

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(*Received in the UK 22 November 1973; Accepted for publication 25 February 1974*)

Abstract—E/F-Indolizidine steroids, which represent a structural unit of Solanum alkaloids, have been synthesized and used to evaluate criteria (IR—Bohlmann bands,[1a-c] —hydrogen bond,[2a-e] NMR,[3a-c] mass spectra,[4] kinetic studies of quaternization with methyl iodide,[5a,b] oxidation with mercuric acetate[6]) for the determination of the stereochemistry of these systems. It was found that the most reliable method is the measurement of the rate constants of quaternization of the bridgehead nitrogen with methyl iodide.

As reported previously,[7a-e] steroids having an indolizidine system attached to ring D can be obtained readily by intramolecular cyclization through high-pressure catalytic hydrogenation. By introduction of an additional Me group in ring F, a partially stabilized or biased model of the related Solanum alkaloids is obtained. This stabilization of the indolizidine system, which is conformationally unstable because of the inversion of the lone pair on the bridgehead N atom, results from the presence of the rest of the steroid molecule.

These model substances were synthesized in a simple, two-stage reaction involving, as the first step, Claisen-Schmidt condensation of estrone methyl ether and 6-methyl-pyridine-2-aldehyde,†[8a-c] and, as the second step, intramolecular cyclization by high pressure catalytic hydrogenation of the resulting product, 17-keto-16-(6-methyl-2-picolinylidene)-3-methoxyestra-1,3,5(10)-triene.

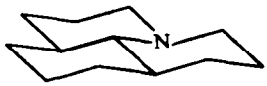
From the resulting mixture, three new isomeric mono-methyl indolizidine steroids were easily isolated by chromatography on a silica-gel column: 5',6',7',8'-tetrahydro-tetrahydro-2'α H-3'α H-9'α-3-methoxyestra-1,3,5(10)-trieno[17,16-b]-5'β-methylindolizidine (**1**), 5',6',7',8'-tetrahydro-2'αH-3'αH-9'αH-3-methoxyestra-1,3,5(10)-trieno[17,16-b]-5'α-methylindolizine (**2**) and 5',6',7',8'-tetrahydro-2'αH-3'αH-9'βH-3-methoxyestra-1,3,5(10)-trieno[17,16-b]-5'-methylindolizine (**3** or **4b**).

Assignments of the configurations and conformations in rings E and F were deduced on the basis of:

(a) Bohlmann bands in IR spectra,
(b) Measurement of the rate constants of quaternization with methyl iodide,
(c) NMR and
(d) Mass spectra.

Theoretical considerations, based on differences in the configurations at positions C-5' and C-9', predict four 16α-H, 17α-H isomers: two belonging to the 9'α-H series (5'α-Me and 5'β-Me) and two in 9'β-H series (5'α-Me and 5'β-Me). (We have assigned the α-H configuration at C-16 and C-17 on the grounds that the hydrogenation of steroids with Pd/C as the catalyst generally occurs from the less hindered α-side of the molecule; it has been shown previously that this assumption is correct[7a-d]). Dreiding models show that one of those four isomers (9'β-H,5'β-CH_3) is difficult to form with E/F rings *trans* fused because of steric hindrance (proximity of the C-18 and C-5' Me groups in which Van der Waals radii overlap).

According to Dreiding models of the possible isomers, rings E and F belonging to the 9'α-H series may be fused only *trans* (a *cis*-ring junction in this series is sterically unlikely because of the strong interactions between ring F and the steroid part of the molecule). Hence, they represent biased models for the *trans*-indolizidine system. (Eliel[9] used the term "biased" to depict systems like tert-butylcyclohexane; "fixed" cyclohexane systems are found, for example, in *trans*-decalin. Our systems **1** and **2** could be regarded as "biased" models would be related, for example, to those synthesized by Johnson *et al.*,[5a] namely t,t-hexahydrojulolidine:



t,t-Hexahydrojulolidine

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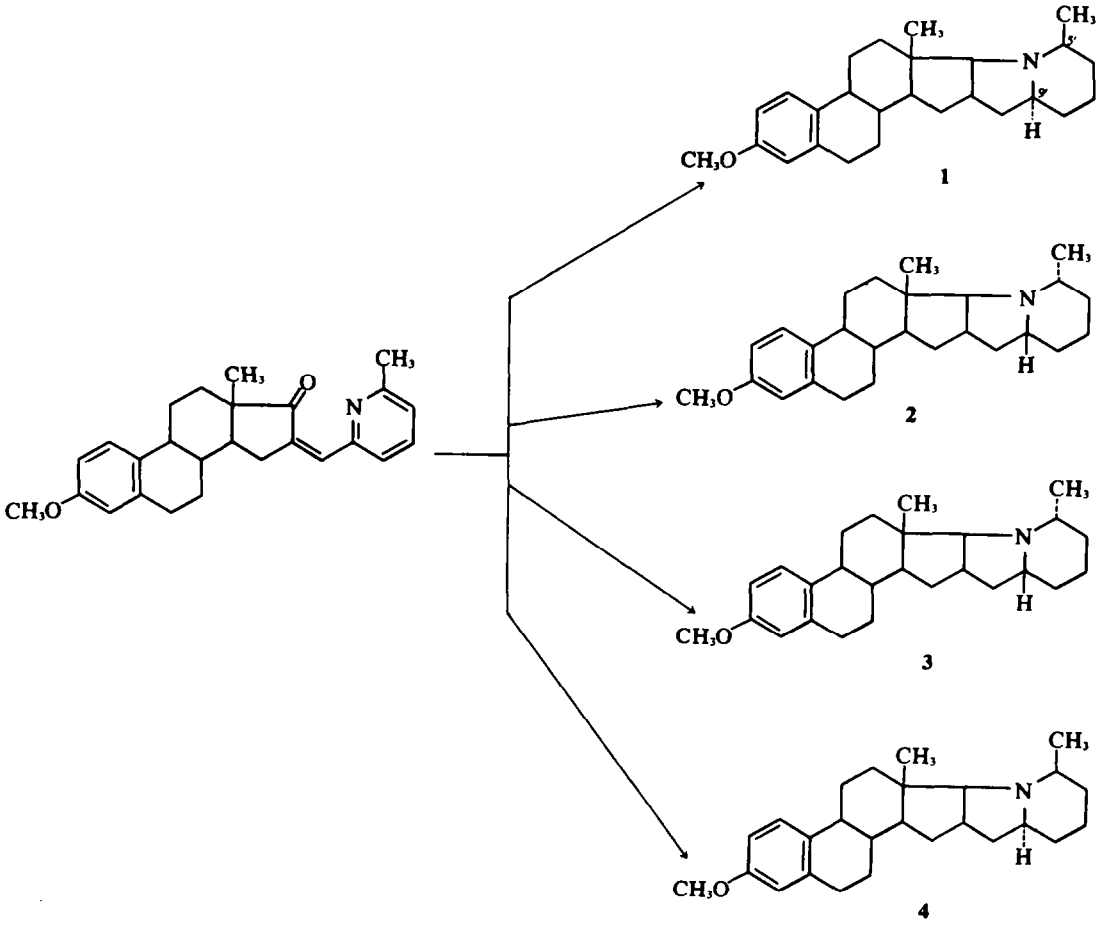
†The main reaction product was a crystalline *trans*-compound obtained in 62·5% yield; the *cis*-isomer was also isolated as oil, by chromatography of the mixture on a silica-gel column, in *ca* 9% yield[7d] (*cis-trans*-stereoisomerism is assigned relative to the C-18 Me group).



However, Dreiding models of the 9′β-H isomer **3** do not reveal any unfavored interactions either in the *trans*- or in the *cis*-E/F ring junction conformation. It is reasonable, therefore, that both conformations exist in solution in rapid equilibrium and interconvert by inversion of the lone electron pair on the bridgehead nitrogen.

Dreiding models of the possible isomers and conformers (Fig 1) also show a very important fact, namely that the electron pair on the N atom in the 9′β-H isomers is less hindered, regardless of whether *trans* or *cis* conformations predominate. The *trans* conformer would be expected to be more basic than the *cis*, but the *cis* conformers should be more basic than either isomer belonging to the 9′α-H series, which represent, as we have seen, stable systems, although they have bridgehead nitrogen. Hence, the 9′β-H isomer should be the most basic among three isolated isomers. On the assumption that the more basic isomer would react more rapidly with methyl iodide in a quaternization process,* we are able to assign the configurations to the isomeric indolizidine steroids that were isolated.

The experimentally obtained rate constants (Table 1) for the quaternization process with methyl iodide of the compounds **1**, **2** and **3** or **4b** are in the ratio 1·00 : 1·34 : 5·16. The data indicate therefore that the 9′β-H configuration corresponds to compound **3** (or **4b**). Compounds **1** and **2** should thus belong to the 9′α-H series.

Table 1. Rate constants (min^{-1}) for the quaternization process with methyl iodide at 25·0°

Compound	k
2	0·00221
2	0·00295
3 (or 4b)	0·0114

*M. Shamma and J. Moss Richey[5b] investigated the stereochemistry of the heterojohimbine alkaloids and found that the kinetics for the formation of the methiodide salts reflected the degree of steric hindrance at the bridgehead nitrogen.

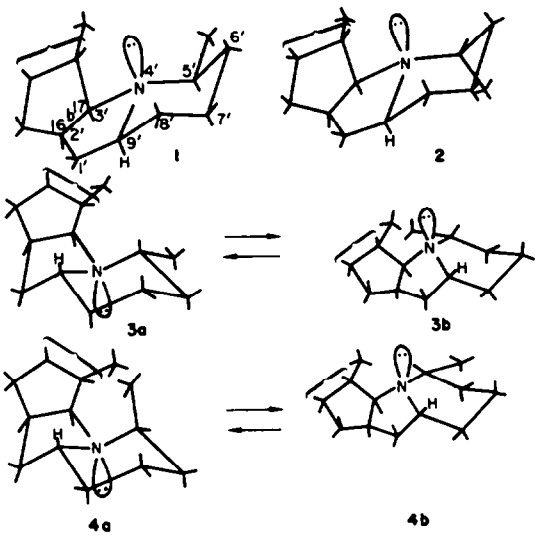


Fig 1. Configurations and conformations of possible isomeric indolizidine steroids **1**, **2**, **3**, and **4**.

We assign the β-configuration to the 5′-Me group (equatorial orientation) in compound **1**. It would be expected to react more slowly than **2** because its lone pair on the bridgehead nitrogen is more hindered compared with the latter which, in turn, is more basic because of the axial orientation of the 5′-Me group.

The Bohlmann bands in IR spectra, and the NMR spectra also fit the proposed structures.

Compounds **1** and **2** exhibit Bohlmann bands in the IR spectra,[1a-c] but compound **3** or **4b** does not. (Bohlmann bands represent complex absorption in the 3·4–3·7 μ (2700–2950 cm^{-1}) region and appear in quinolizidine[1a-c] and indolizidine[2c] systems having at least two H atoms α to the nitrogen, which are in a *trans* diaxial relationship to the lone pair on nitrogen). Bohlmann bands in IR spectrum of compound **1** may be explained by the presence of three H atoms which fulfill Bohlmann conditions (9′α-H, 17α-H and 5′α-H), and in compound **2** by the presence of two corresponding H atoms (9′α-H and 17α-H). The lack of Bohlmann bands* in the IR spectrum of compound **3** (or **4b**) reflects the *cis* junction of rings E and F. By contrast, the *trans* conformation of **3** fulfills the Bohlmann criteria.†

The NMR spectra of the isolated isomers also support the *cis* junction of rings E and F in compound **3** (or **4b**). They are also in agreement with the conclusion that compounds **1** and **2** both belong to the same (9′α-H) series. Thus, the position of the signals for the C-18 Me group in NMR spectra of compounds **1** and **2** are *exactly* the same (δ 0·783) and indicate that these compounds belong to the same series. On the other hand the signal for C-18 Me group in **3** falls upfield at δ 0·72. In the δ 1·4–1·62 region in NMR spectrum of **1** there is a split signal corresponding to 6 protons. This signal appears to be due to the protons in ring F adjacent to the C-6′, C-7′ and C-8′ atoms, and the splitting of this signal indicates that the system having these protons is conformationally fixed. By contrast, the flexible *cis* junction of rings E and F in **3** (or **4b**) causes an unsplit signal at δ 1·27–1·44 for the corresponding protons, thus indicating a flexible system.

We suggest that caution should be exercised in the use of NMR results in assignment of stereochemistry on bridgehead nitrogen systems. Moynehan's example[10] proves this, as well as many others, in which NMR method is not very useful because of many complicating factors.[3a-c]

The mass spectra of compounds **1**, **2** and **3** (or **4b**) are very similar. The only difference is seen in the relative abundance of the corresponding peaks. They show the same molecular weight (379) corresponding to the same molecular formula $C_{26}H_{37}ON$, and the same base peak at 150 mass units indicating that the fragmentation path is very similar to those of solanidine[4] (α-cleavage).

Other possible methods for the determination of the stereochemistry on bridgehead nitrogen are the measurements of pK_a values,[5b] the oxidation reaction with mercuric acetate,[6] Aaron's IR method,[2a-c] Grob's kinetic method,[11] and Eschenmoser's kinetic interconversion method.[12a-d]

The differences in pK_a values are not significant enough in systems with similar basicity. Also, some systems fail to react with mercuric acetate because of the steric hindrance of the bridgehead hydrogen rather than because of the unfavorable *cis* ring junction. Aaron's IR method is applicable only in systems having OH group. Grob's kinetic method can be applied only to esters of α-amino-ketoximes. Eschenmoser's method deals with systems having a high energetic barrier for inversion of the lone pair on the N atom, thus permitting isolation of the corresponding *isomers* at the room temperature.

It appears therefore that the method of determining the rate constants for quaternization with methyl iodide is the most reliable one, because it gives, especially in our case, more information about difference in configuration than the other methods. Also, this method permits the distinction between **1** and **2**, which is not possible using Bohlmann's method.

Finally, it can be pointed out that the stereochemical considerations concerning the indolizidine sys-

*In some cases the low intensity of these bands makes the method less reliable.[3a]

†One exception to Bohlmann's rule is found, namely one of the two isomeric 4-methylquinolizidines exhibit Bohlmann bands, but the second does not, although both systems are *trans*-fused and fulfill the Bohlmann conditions. Moynehan *et al.*[10] attempted to explain the absence of Bohlmann bands by a *cis*-ring junction in the corresponding isomer on the basis of an NMR analysis of 4-methylquinolizidine, as well as its methiodide salt. That this interpretation is incorrect was shown by Johnson *et al.*[5a] by the kinetic method of quaternization with methyl iodide.

tem and the methods for its determination can be also applied at quinolizidine and pyrrolizidine systems, structural units of Rauwolfia[13a] and Senecio[13b] alkaloids, respectively.

EXPERIMENTAL

M.ps (uncorrected) were determined on a Büchi apparatus.

The column chromatography was performed on silica-gel (Merck, 0·2–0·5 mm). MN-Silica-gel G was used for TLC which was carried out with benzene–EtOAc (7:3); the detection was effected by spraying with a mixture of 50% H_2SO_4 (100 ml) and anisaldehyde (1 g) and by heating at 100°.

IR spectra (KBr pellets or $CHCl_3$ soln) were recorded on a Perkin-Elmer 337-G apparatus. NMR spectra were obtained at 60 MC with a Varian Associates A-60 A apparatus in $CDCl_3$ soln using TMS as internal standard. Chemical shifts (δ) are given in ppm, and coupling constants in Hz; symbols *s*, *d* and *m* indicate singlet, doublet and multiplet, respectively; the numbers in parentheses represent the number of protons of the corresponding signal). Mass spectra were obtained on Varian-MAT, CH-5 apparatus.

The conductivity was measured with the conventional apparatus which consisted of a conductivity cell with platinum electrodes, a thermostat which maintained the temperature at 25·00 ± 0·02° (Ultra-thermostat, Type U 1, VEB, Prüfgeräte-Werk Medigen, DDR), and the conductometer MA 5961 "Iskra", Kranj.

Hydrogenation of 17 - *keto* - 16 - (6 - *methyl* - 2 - *picolinylidene*) - 3 - *methoxyestra* - 1,3,5,(10) - *triene under the conditions of the reductive cyclization*

17 - Keto - 16 - (6 - methyl - 2 - picolinylidene) - 3 - methoxyestra - 1,3,5,(10) - triene (5 g) was dissolved in dioxane (50 ml), 5% Pd/C (7 g) was added and the mixture was placed in an autoclave. The hydrogenation was performed during 24 h at the average temp and pressure of 80° and 80 atm. The autoclave was then washed out with $CHCl_3$, the catalyst removed by filtering through Hyflo Supercel and the solvents evaporated. By repeated column chromatography of this extract, three substances were isolated which gave yellow spots on the TLC: product **1** (170 mg) having the highest R_f value; compound **1** was obtained by elution with benzene, the product **2** (410 mg) having lower R_f value than **1**; **2** was obtained by elution with benzene–chloroform 50:50, and the product **3** (80 mg) having the lowest R_f value; **3** was obtained by elution with $CHCl_3$–EtOAc 50:50 and EtOAc.

The isolated products **1**, **2** and **3** have the following physical and spectral characteristics:

Compound **1**. m.p. = 147·5–148·5°. $[\alpha]_D^{20} = 71·2°$ (c = 0·94 g/100 ml; $CHCl_3$).

IR spectrum: ν 3000, 2970, 2950, 2930, 2910, 2890, 2870, 2850, 2820, 2760, 1595, 1560, 1480, 1430, 1420, 1365, 1350, 1330, 1296, 1278, 1253, 1235, 1224, 1210, 1193, 1144, 1126, 1115, 1096, 1078, 1066, 1050, 1035, 996, 975, 955, 925, 890, 870, 865, 845, 816, 808, 782, 725 cm^{-1}.

NMR spectrum: δ 0·783(s) (3, C-18–CH_3), 0·95 (d) (J = 7) (3, C-5′ –CH_3), 1·4–1·62 (6H, C-6′, C-7′ and C-8′H), 2·17 (s) (1H), 3·783 (s) (3, C-3 –OCH_3), 6·65–7·37 (m) (3, the protons of the ring A).

Mass spectrum (the numbers in parentheses represent the relative abundance in per cent): *m/e* 122 (3·4), 136 (12·2), 150 (B) (100), 189, 190, 364 (8·1), 379 (M^+) (17·2).

The rate constant of the quaternization with methyl iodide at 25·0° $k_1 = 0·00221$ min^{-1}. (Found: C, 82·05; H, 9·75; N, 4·10. $C_{26}H_{37}ON$ requires: C, 82·27; H, 9·83; N, 3·69%).

Compound **2**. m.p. = 128–129°. $[\alpha]_D^{20} = 96·8°$ (c = 1·00 g/100 ml; $CHCl_3$).

IR spectrum: ν 3000, 2980, 2940, 2920, 2850, 2829, 2770, 2700, 2570, 1600, 1560, 1495, 1445, 1380, 1370, 1360, 1350, 1310, 1295, 1275, 1245, 1238, 1220, 1195, 1180, 1170, 1145, 1128, 1115, 1100, 1082, 1060, 1035, 975, 960, 925, 892, 870, 842, 810, 780, 722 cm^{-1}.

NMR spectrum: δ 0·783 (s) (3, C-18 –CH_3), 1·033 (d) (J = 7) (3, C-5′–CH_3), 3·783 (s) (3, C-3–OCH_3), 6·62–7·25 (m) (3, the protons of the ring A).

Mass spectrum (the numbers in parentheses represent relative abundance in per cent): *m/e* 122 (3·1), 136 (8·8), 150 (B) (100), 189, 190, 364, (2·0), 379 (M^+) (16·6).

The rate constant of the quaternization with MeI at 25·0° $k_2 = 0·00295$ min^{-1}. (Found: C, 82·32; H, 9·86; N, 3·83. $C_{26}H_{37}ON$ requires: C, 82·27; H, 9·83; N, 3·69%).

Compound **3**. m.p. = 92–94° (129–131°). $[\alpha]_D^{20} = 51·4°$ (c = 0·4475 g/100 ml; $CHCl_3$).

IR spectrum: ν 2960, 2920, 2850, 2820, 1595, 1560, 1490, 1450, 1440, 1360, 1340, 1330, 1320, 1305, 1272, 1250, 1220, 1208, 1200, 1190, 1175, 1155, 1140, 1095, 1066, 1047, 1033, 1015, 996, 985, 975, 965, 945, 930, 920, 900, 890, 858, 850, 833, 782, 755, 745, 733, 720 cm^{-1}.

NMR spectrum: δ 0·72 (s) (3, C-18–CH_3), 1·08 (d) (J = 7) (3, C-5′ –CH_3), 1·27–1·44 (6H, C-6′, C-7′ and C-8′H), 1·63–1·72 (2H), 3·783 (s) (3, C-3 –OCH_3), 6·67–7·3 (m) (3, the protons of the ring A).

Mass spectrum: (the numbers in parenteses represent the relative abundance in per cent): *m/e* 122 (3·2), 136 (8·6), 150 (B) (100), 364 (3·7), 379 (M^+) (17·6).

The rate constant of the quaternization with methyl iodide at 25·0° $k_3 = 0·0114$ min^{-1}. (Found: C, 82·51; H, 10·08; N, 3·76. $C_{26}H_{37}ON$ requires: C, 82·27; H, 9·83; N, 3·69%).

Acknowledgements—The authors are grateful to the Research Fund of the Serbian Academy of Sciences and Arts for financial support, and to Mrs. Dr. R. Tasovac for the elemental microanalyses and Dr. D. Jeremić for NMR and Mass spectra (which were measured in our Instrumental Division).

We gratefully acknowledge the editorial assistance of Professor A. W. Burgstahler of the University of Kansas in the preparation of this manuscript.

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