Three Novel Glycodienoid Alkaloids from Erythrina lysistemon

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THREE NOVEL GLYCODIENOID ALKALOIDS FROM *ERYTHRINA LYSISTEMON*

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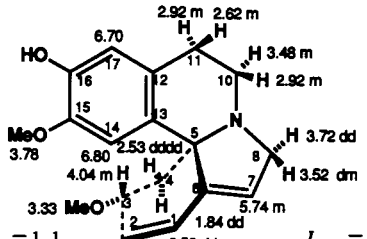
ABSTRACT.—Three new glycodienoid alkaloids, (+)-11β-methoxyglucoerysodine [**3**], (+)-11β-methoxyglucoerysovine [**4**], and (+)-rhamnoerysodine [**5**], have been isolated from Egyptian-grown *Erythrina lysistemon*. The known (+)-glucoerysodine [**2**] was also obtained, and ^{1}H- and ^{13}C-nmr values are presented for the dienoid and glycoside portions. A reversal of proton assignments for H-1 and H-2 in all previously described dienoids is noted.

Since the original identification of *Erythrina* alkaloids by Folkers and Major (1) in 1937, this family of compounds has grown to include some 95 members, many of which have been shown to possess curare-like activity (1,2). This neuromuscular blocking property has prompted our study of the seeds of Egyptian-grown *Erythrina lysistemon* Hutchinson (Leguminosae). We were able to reisolate five previously described dienoids, namely (+)-erysodine [**1**], (+)-erythristemine, (+)-erysotrine, (+)-erythravine, and (+)-erysotrine *N*-oxide, all of which were readily identified through nmr and mass spectral comparisons (3). Of greater import, however, was the isolation of three novel glycosides, (+)-11β-methoxyglucoerysodine [**3**], (+)-11β-methoxyglucoerysovine [**4**], and (+)-rhamnoerysodine [**5**]. The known (+)-glucoerysodine [**2**] was also reisolated and characterized by nmr spectroscopy. Isomers **3** and **4** were analyzed as a mixture because they could not be separated chromatographically.

The ^{1}H-nmr spectral characteristics for each of the new alkaloids will be described in detail in the discussion that follows. It should be pointed out immediately that detailed ^{1}H and ^{13}C data were employed to establish the identities of compounds **1** through **5**. These led to the reversal of the ^{1}H chemical shift assignments for H-1 and H-2 of (+)-erysodine [**1**] in particular, and consequently for all the commonly accepted H-1 and H-2 values of *Erythrina* dienoids in general (4). Additionally, the ^{13}C chemical shifts of C-2 and C-13 in the known (+)-glucoerysodine [**2**] have been reversed based upon heteronuclear correlation experiments (5). The ^{1}H and ^{13}C assignments for the glucose moiety of this alkaloid are now reported for the first time around structures **2a** and **2b**, respectively.

Throughout the analysis of the dienoid alkaloids which we obtained from *E. lysistemon* as well as those which we found in a related study of *Erythrina caffra*,[1] our nmr nOe studies repeatedly showed a strong mutual enhancement between H-3 (usually found near δ 4.0) and the doublet of multiplets resonating around δ 6.0 (see Experimental) which had previously been assigned to H-1(4). Specifically, perusal of structure **1** made it apparent that H-1 and H-3 are located too far apart for a large nOe to be possible. Rather, proton assignments about the perimeter of ring A can best be explained by reassigning the δ 6.00 signal to H-2 and the δ 6.58 doublet of doublets to H-1 as shown around structure **1**. The H-7 signal (δ 5.74) shows sizeable nOe enhancements with the doublet of doublets at δ 6.58, thus further establishing the identity of the latter as H-1.

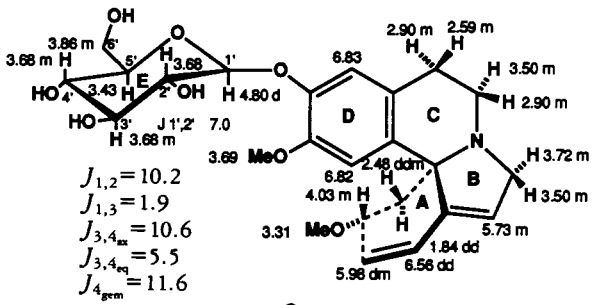
[1]M.E. Amer, S. El-Masry, M. Shamma, and A.J. Freyer, unpublished results.



$J_{4_{eq},7}=1.1$
$J_{4_{eq},2}=1.1$
$J_{4_{ax},3}=10.5$
$J_{4_{eq},3}=5.6$
$J_{4_{gem}}=11.5$

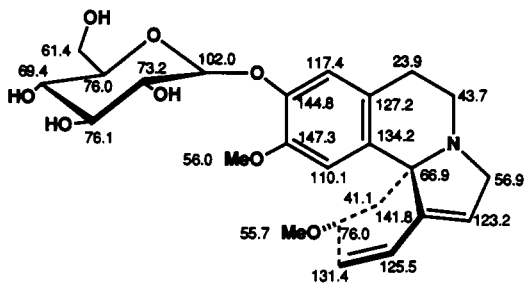
1

$J_{8_{gem}}=14.5$
$J_{8\alpha,7}=3.0$
$J_{1,2}=10.1$
$J_{1,3}=2.3$



$J_{1,2}=10.2$
$J_{1,3}=1.9$
$J_{3,4_{ax}}=10.6$
$J_{3,4_{eq}}=5.5$
$J_{4_{gem}}=11.6$

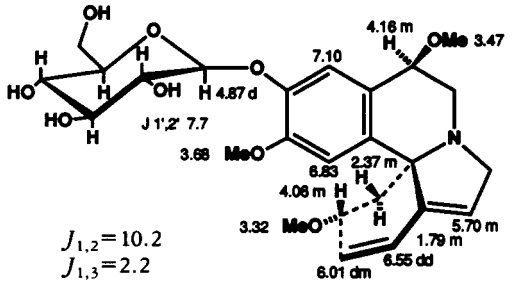
2a



2b

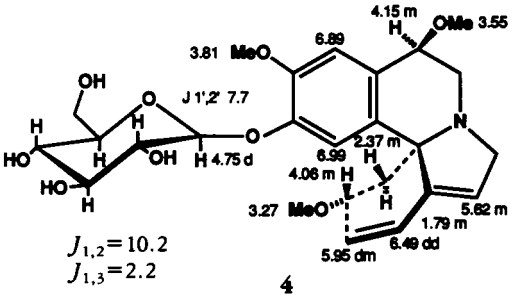
Additionally, a ^{13}C-^{1}H correlated spectrum of (+)-erysodine [**1**] confirmed that H-1 (δ 6.58) was attached to C-1 (δ 125.5) and that H-2 (δ 6.00) was directly bonded to C-2 (δ 131.4) (3). The present reversal in H-1 and H-2 assignments also applies to all of the known *Erythrina* dienoid alkaloids.

The first glycodienoid that we isolated proved to be the known (+)-glucoerysodine [**2**] (5). The ^{1}H-nmr chemical shifts appear about structure **2a**, and the ^{13}C values are shown around **2b**. The two methoxyl proton peaks, as well as the olefinic resonances, parallel those for the dienoid (+)-erysodine [**1**]. Even the aliphatic protons appear to duplicate the chemical shifts and coupling constants of their counterparts in **1**. Although H-14 (δ 6.82) has virtually the same chemical shift as in (+)-erysodine [**1**], H-17 is located 0.13 ppm further downfield at δ 6.83 in the glycoside. Additionally, a number of overlapping multiplets appear between δ 3.86 and δ 3.43. along with a distinct doublet at δ 4.80. These signals represent the glucose moiety which is presumably attached through an oxygen to C-16. This glucoside must have a β attachment, since H-1′ (δ 4.80) exhibits a 7.0 Hz diaxial coupling with H-2′ (δ 3.68). A 2D NOESY ex-



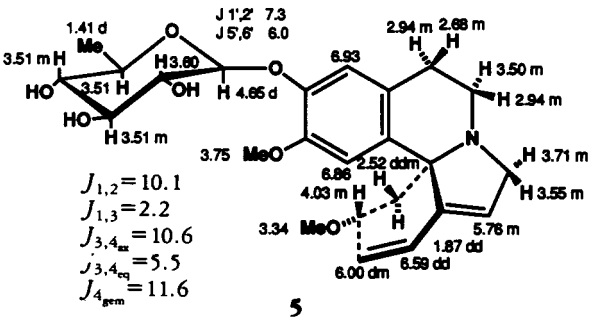
$J_{1,2}=10.2$
$J_{1,3}=2.2$

3



$J_{1,2}=10.2$
$J_{1,3}=2.2$

4



$J_{1,2}=10.1$
$J_{1,3}=2.2$
$J_{3,4_{ax}}=10.6$
$J_{3,4_{eq}}=5.5$
$J_{4_{gem}}=11.6$

5

periment confirmed these conclusions. One-bond and long range ^{13}C-^{1}H correlated spectra aided in the carbon assignment as seen in **2b**. Of particular interest is a three-bond coupling between H-1′ (δ 4.80) and C-16 (δ 144.8), confirming C-16 as the site of attachment of the sugar moiety. In the original report (5) of the carbon data of (+)-glucoerysodine, the chemical shifts of C-13 (δ 134.2) and C-2 (δ 131.4) were reversed, but they now appear corrected around structure **2b**.

Acetylation of (+)-glucoerysodine [**2**] produced an increase in mol wt from *m/z* 461 to *m/z* 629. The mass change indicates four hydroxyl groups were acetylated, as one would expect for a glucoside.

Our next two glycosides were previously unknown and had to be analyzed as a mixture because it was not possible to separate them chromatographically. Fortunately, the aromatic and olefinic protons were well separated as were the methoxyls and anomeric hydrogens.

The nmr data for the more abundant compound of the mixture, (+)-11β-methoxyglucoerysodine, is displayed about structure **3**. The chemical shifts and coupling constants were generally reminiscent of (+)-glucoerysodine [**2**] with the exception of H-17, which now appeared 0.27 ppm further downfield at δ 7.10. An additional aliphatic methoxyl peak was present at δ 3.47, and a narrow multiplet appeared at δ 4.16 due to

H-11. NOe studies showed mutual enhancements between the downfield H-17 (δ 7.10), the aliphatic methoxyl at δ 3.47, and the narrow H-11 multiplet at δ 4.16 suggesting their close proximity to each other as shown in **3**. The glycoside moiety closely resembled that of (+)-glucoerysodine [**2**], even to the 7.7 Hz diaxial coupling of H-1′ (δ 4.87). Attaching the β-glucoside to C-16 of the dienoid was prompted by strong reciprocating nOe enhancements between H-1′ (δ 4.87) and H-17 (δ 7.10).

Acetylation of glucoside **3** produced an increase in mol wt of 168 amu, thus confirming the existence of four initial hydroxyl sites.

The ^{1}H-nmr values for the minor component of the mixture, (+)-11β-methoxyglucoerysovine, are shown around structure **4**. They are very similar to those of the main component **3**, with the exception of the chemical shifts of the aromatic and methoxyl singlets of ring D. NOe irradiation of the H-14 signal (δ 6.99) produced a 17% enhancement of the anomeric doublet (δ 4.75) and a 30% increase of H-3 (δ 4.06). Alternatively, H-17 (δ 6.89) showed mutual nOe enhancements with 16-MeO (δ 3.81), 11-MeO (δ 3.55), and H-11 (δ 4.15). Reported literature values for H-17 and 16-MeO for (+)-erysovine are δ 6.84 and δ 3.87, respectively, and these values compare well with those presently found for (+)-11β-methoxyglucoerysovine [**4**].

The last glycoside that we isolated from *E. lysistemon* was (+)-rhamnoerysodine [**5**]. The nmr values for the dienoid portion of this alkaloid closely resembled those of (+)-glucoerysodine [**2**]. There were, however, noticeable changes in the peaks due to the glycoside moiety, including the appearance of a three-proton methyl doublet at δ 1.41 with a 6.0 Hz coupling and an accompanying upfield shift of the distinctive anomeric proton H-1′ to δ 4.65.

Decoupling the H-5′ proton (δ 3.51) caused the H-6′ methyl doublet (δ 1.41) to collapse. Similarly, irradiation of H-2′ (δ 3.60) effected a simplification of the H-1′ doublet (δ 4.65). NOe enhancements established the network between H-14 (δ 6.86), 15-MeO (δ 3.75), and H-3 (δ 4.03), allowing for their definite assignments. NOe irradiation of H-1′ (δ 4.65) enhanced H-17 (δ 6.93) by 16%, suggesting that the rhamnoside moiety was connected to C-16 through an oxygen atom.

The acetylated product yielded a parent ion that was 126 amu higher than that of **5**, indicating the presence of only three hydroxyl sites in the original molecule.

Some general conclusions can be drawn concerning the nmr shifts of *Erythrina* alkaloid glycosides. When one is dealing with (+)-erysodine glycosides, as represented by **2**, **3**, and **5**, the ^{1}H chemical shift of H-14 remains moderately close to the base value of δ 6.80 while H-17 varies widely, depending upon the type of glycoside attached to C-16 and also on the nature of the oxygenated substituent at C-11. Conversely, when the glycoside is attached to C-15, as is the case with **4**, one can expect H-17 to resonate near the δ 6.84 base value exhibited by (+)-erysovine. However, these types of assignments cannot be made safely without careful nOe difference or NOESY studies. Because the location of H-3 (δ 4.0) is constant throughout the *Erythrina* series, one can fix the location of H-14 by their large mutual nOe enhancements. The anomeric proton H-1′, which is also very characteristic and well separated from the other peaks, can be expected to display large nOe interactions with the aromatic hydrogen on the carbon adjacent to the site of the glycosidic linkage.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Optical rotations are measured at 25° ^{1}H-nmr spectra were recorded on a Bruker AM-500 (^{13}C at 125 MHz) in $CDCl_3$. ^{13}C/^{1}H correlation data and COLOC experiments were collected in the inverse mode. NOe's were calculated as % maximum possible enhancement, so that a maximum 0.5 enhancement corresponded to 100% nOe. Mass spectral data was collected on a Kratos MS9/50 using electron impact ionization. Cc was on Merck Kieselgel 60. Tlc was on Merck Si gel glass plates, 0.25 mm thick.

PLANT COLLECTION.—The fruits of *E. lysistemon* were collected in May 1989 from trees growing near Alexandria, Egypt. A voucher specimen was deposited in the Department of Pharmacognosy, Faculty of Pharmacy, University of Alexandria.

EXTRACTION AND FRACTIONATION.—Seeds (2.5 kg) from the fruits of *E. lysistemon* were ground, defatted with light petroleum ether at 50°, and extracted with EtOH. The solvent was evaporated to leave a semi-solid extract that was placed on a Si gel column. Four fractions [light petroleum ether, $CHCl_3$, $CHCl_3$-MeOH (1:1), and MeOH] were collected. Cc of the $CHCl_3$-soluble extract afforded (+)-erythristemine (8 mg) and (+)-erysotrine (8 mg). The two more polar extracts were subjected to cc, eluting with $CHCl_3$ gradually enriched with MeOH. Products of this separation were (+)-erysotrine (4 mg), (+)-erythristemine (4 mg), (+)-erysodine [**1**] (3 mg), (+)-erythravine (6 mg), and (+)-erysotrine *N*-oxide (5 mg), in addition to the glycosides (+)-glucoerysodine [**2**] (15 mg), (+)-11β-methoxyglucoerysodine [**3**] (8 mg), (+)-11β-methoxyglucoerysovine [**4**] (4 mg), and (+)-rhamnoerysodine [**5**] (6 mg). All samples are amorphous.

(+)-ERYSODINE [**1**].—Uv λ max (MeOH) 219, 236, 238, 283 nm (log ε 3.90, 3.96, 3.94, 3.35); eims *m/z* (%) $[M]^+$ 299 (50), 284 (43), 269 (26), 268 (100), 266 (15), 253 (10), 241 (10), 228 (8), 215 (16); $[\alpha]_D$ +245° (*C* = 0.1, $CHCl_3$). Principal nOe's 15-MeO to H-14 (36), H-3 to H-14 (23), H-3 to 3-MeO (13), H-3 to H-4_{eq} (8), H-3 to H-2 (14), H-8β to H-7 (6), H-8α to H-7 (15), H-10_{ax} to H-4_{eq} (6), H-4_{eq} to H-10_{ax} (4), H-11_{eq} to H-17 (10), H-11_{ax} to H-17 (3).

(+)-GLUCOERYSODINE [**2**].—Uv λ max (MeOH) 205, 225, 281 nm (log ε 4.26, 4.03, 3.39); eims *m/z* (%) $[M]^+$ 461 (26), 430 (4), 299 (100), 284 (56), 268 (61), 254 (8), 241 (14), 214 (9), 167 (4), 151 (4), 130 (8); $[\alpha]_D$ +101° (*c* = 1.55, $CHCl_3$). Nmr experiments conducted include NOESY, homonuclear decoupling, gated spin echo, $^{13}C/^1H$ correlation, and COLOC experiments.

(+)-2′,3′,4′,6′-TETRAACETYLGLUCOERYSODINE.—Alkaloid **2** (1 mg) was treated with Ac_2O in pyridine at room temperature to provie (+)-2′,3′,4′,6′-tetraacetylglucoerysodine: eims *m/z* (%) $[M]^+$ 629 (1), 598 (2), 446 (3), 299 (2), 284 (2), 266 (10), 250 (3), 241 (2), 169 (12), 127 (5), 43 (100).

(+)-11β-METHOXYGLUCOERYSODINE [**3**].—Uv λ max (MeOH) 208, 226, 287 nm (log ε 4.21, 4.02, 3.18); eims *m/z* (%) $[M]^+$ 491 (5), 459 (20), 329 (42), 314 (18), 298 (100), 282 (53), 266 (78), 250 (22), 234 (18), 213 (18), 167 (14); $[\alpha]_D$ +73° (*c* = 0.42, $CHCl_3$). Principal nOe's H-17 to H-1′ (13), H-1′ to H-17 (17), H-17 to H-11 (10), H-11 to H-17 (17), H-17 to 11-MeO (7), 11-MeO to H-17 (4), H-14 to H-3 (18), H-3 to H-14 (20), H-14 to 15-MeO (23), 15-MeO to H-14 (28), H-3 to H-2 (18), H-2 to H-3 (11), H-3 to 3-MeO (14), 3-MeO to H-3 (15), H-3 to H-4_{eq} (6), H-4_{eq} to H-3 (14),3-MeO to H-2 (11), H-2 to 3-MeO (9), H-4_{eq} to 3-MeO (4), H-2 to H-1 (30), H-1 to H-2 (16), H-1 to H-7 (11), H-7 to H-1 (16), H-8α to H-7 (19), H-10_{ax} to H-11 (16), H-10_{eq} to 11-MeO (10), 11-MeO to H-10_{eq} (9), 11-MeO to H-11 (23), H-11 to 11-MeO (19).

(+)-2′,3′,4′,6′-TETRAACETYL-11β-METHOXYGLUCOERYSODINE.—Alkaloid **3** (2 mg) was treated with Ac_2O in pyridine at room temperature to provide (+)-2′,3′,4′,6′-tetraacetyl-11β-methoxyglucoerysodine: eims *m/z* (%) $[M]^+$ 659 (28), 644 (3), 628 (43), 593 (7), 446 (6), 329 (32), 314 (20), 297 (93), 282 (35), 271 (30), 263 (100), 248 (34), 239 (21), 220 (35).

(+)-11β-METHOXYGLUCOERYSOVINE [**4**].—Principal nOe's H-17 to 16-MeO (22), 16-MeO to H-17 (31), H-17 to H-11 (14), H-11 to H-17 (10), H-17 to 11-MeO (3), 11-MeO to H-17 (3), H-14 to H-3 (30), H-3 to H-14 (8), H-14 to H-1′ (17), H-1′ to H-14 (16), H-3 to H-2 (13), H-2 to H-3 (13), H-3 to 3-MeO (7), 3-MeO to H-3 (14), 3-MeO to H-2 (8), H-2 to 3-MeO (3), H-2 to H-1 (15), H-1 to H-2 (14), H-1 to H-7 (9), H-7 to H-1 (15), H-8α to H-7 (16), H-11 to 11-MeO (14).

(+)-2′,3′,4′,6′-TETRAACETYL-11β-METHOXYGLUCOERYSOVINE.—Alkaloid **4** (2 mg) was treated with Ac_2O in pyridine at room temperature to provide (+)-2′,3′,4′,6′-tetraacetyl-11β-methoxyglucoerysovine.

(+)-RHAMNOERYSODINE [**5**].—Uv λ max (MeOH) 208, 226, 258, 279 nm (log ε 4.30, 4.22, 3.63, 3.51); eims *m/z* (%) $[M]^+$ 445 (5), 299 (59), 284 (37), 268 (100), 254 (11), 241 (15), 228 (9), 215 (14), 130 (6); $[\alpha]_D$ +83° (*c* = 0.13 $CHCl_3$). Principal nOe's H-17 to H-1′ (11), H-1′ to H-17 (16), H-14 to H-3 (18), H-3 to H-14 (17), H-14 to 15-MeO (19), 15-MeO to H-14 (27), H-3 to H-2 (19), H-3 to 3-MeO (11), H-3 to H-4_{eq}(8).

(+)-2′,3′,4′-TRIACETYLRHAMNOERYSODINE.—Alkaloid **5** (2 mg) was treated with Ac_2O in pyridine at room temperature to provide (+)-2′,3′,4′-triacetylrhamnoerysodine: eims *m/z* (%) $[M]^+$ 571 (9), 299 (60), 284 (33), 268 (100).

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