NEW ACRIDONE DERIVATIVES OBTAINED BY HEATING THE HCL SALT OF ACRONYCINE[1]

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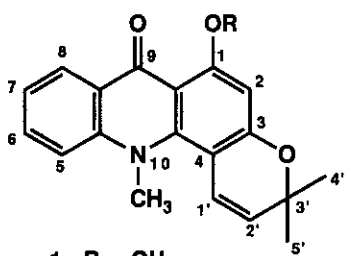
Abstract - By heating the HCl salt of acronycine (**1**), the new acridone derivatives (**6**, **9**, **13** and **15**) were obtained together with noracronycine (**2**), dihydronoracronycine (**3**), 1,3-dihydroxy-10-methylacridone (**4**) and (**5**). Compound (**5**), one of the main products of this reaction, is a linear type dihydro derivative of **2**, compound (**9**) is a furanoacridone derivative with a geminal methyl group and compound (**15**) is a derivative of **5** with an additional C_5 unit.

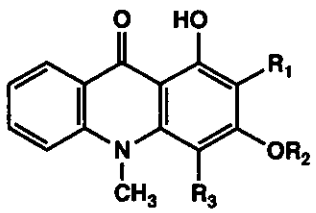
Acronycine (**1**), an alkaloid isolated in 1948 from the bark of the Australian scrub ash *Baurella simplicifolia* (Endl.) Hartley (syn. *Acronychia baueri* Scott) (Rutaceae), was one of



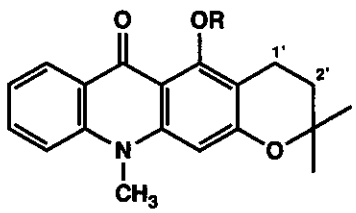
1 R = CH_3
2 R = H
3 R = H, 1',2'-H_2
11 R = CH_3, 1',2'-H_2

the first acridone alkaloids isolated from natural sources.[2] Subsequently, it was shown that **1** possessed a broad spectrum of antitumor activity in experimental animals.[3,4] In spite of this, relatively little is known of the chemistry or mode of action of acridone alkaloids.[5,6] Because of our interest in the development of acronycine (**1**) and/or its derivatives as antineoplastic agents, we have been investigating their chemical and biological properties.[1,5,6]

In the course of these experiments, it was reported that noracronycine (**2**) was obtained in 65% yield when the HCl salt of acronycine (**1**) was heated at 140°C for 1 hr.[7] When the reaction mixture was heated at higher temperature, noracronycine (**2**) was obtained in only trace amounts and a complex mixture resulted. Careful chromatographic separation afforded eight reaction products. Four of these compounds were identified as noracronycine (**2**), dihydronoracronycine (**3**), 1,3-dihydroxy-10-methylacridone (**4**) and dihydronorisoacronycine (**5**), respectively, and **5** was one of the main reaction products. In earlier studies,[6] dihydronorisoacronycine (**5**) had been derived from acronycine (**1**) through four steps.[6]



4 $R_1 = R_2 = R_3 = H$
6 $R_1 = CH_3$, $R_2 = R_3 = H$
7 $R_1 = CH_3$, $R_2 = Ac$, $R_3 = H$
8 $R_1 = H$, $R_2 = Ac$, $R_3 = CH_3$

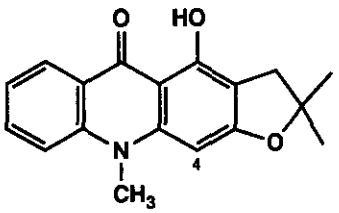


5 R = H
12 R = CH_3

One of the reaction products (**6**) was sparingly soluble in any solvents and was acetylated with Ac_2O/pyridine to afford **7**. The uv spectrum of **7** (λ_{max} 264, 310, 408) indicated that this compound was an acridone derivative and a molecular ion at *m/z* 297 was observed in the EIms. On the other hand, in the ^1H nmr spectrum of **7**, in addition to a set of four aromatic signals coupled to each other (δ_H 7.31, 7.50, 7.75, 8.49), a singlet aromatic signal at δ_H 6.62, a *N*-Me signal at δ_H 3.81, a 3 proton signal attributed to an acetyl moiety (δ_H 2.39), a hydrogen bonded one proton signal at δ_H 14.98 and a methyl signal at δ_H 2.12 were observed. From its chemical shift, this methyl moiety was estimated to be attached directly to the aromatic moiety. From these observations, the structure of this product was elucidated as either **7** or **8**. To determine the structure of this compound nOe difference experiments were conducted. Thus, when the *N*-Me signal (δ_H 3.81) was irradiated, an 18% nOe was observed at δ_H 6.62 (1H, s), and a 4% nOe was observed at δ_H 3.81 when the aromatic signal at δ_H 6.62 was irradiated. No nOe was observed at δ_H 3.81 when the resonance at δ_H 2.12 was irradiated. These results strongly support the structure (**7**) for this compound, and that the structure of the compound obtained from the reaction was **6**.

The second compound (**9**) was obtained as a yellow powder, and its uv spectral data (λ_{max} 274, 330, 392) indicated that it possessed an acridone chromophore. A molecular ion at *m/z* 295 was observed in the EIms of compound (**9**), which is 14 amu smaller than that of dihydronoracronycine (**3**) and dihydronorisoacronycine (**5**). The ^1H nmr spectrum of this compound was similar to those of **3** and **5**, except that a singlet methylene signal at δ_H 3.07

(2H, s) was observed instead of a set of two methylene signals coupled with each other.[6] Thus, it was elucidated that this compound possessed either structure (**9**) or (**10**). Again, to distinguish between these structures, nOe experments were conducted. When the signal at δ_H 3.80 (3H, s) attributed to the *N*-Me was irradiated, a 16% nOe was observed in the resonance at δ_H 6.28 (1H, s, H-4). On the other hand, when the singlet signal at δ_H 6.28 (1H, s, H-4) was irradiated, a 6.5% nOe was observed at δ_H 3.80. Thus, it was concluded that this reaction product possessed the linear structure (**9**).



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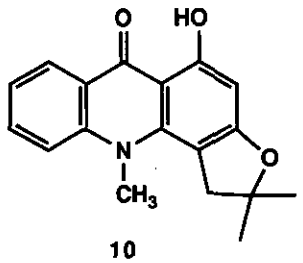

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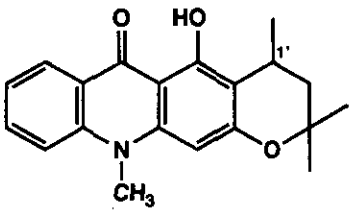
The third compound (**13**) was obtained as an orange oil. The acridone chromophore was elucidated from its uv spectral data, and a molecular ion at *m/z* 323 was observed in the EIms, which was the same as that of dihydroacronycine (**11**) and dihydroisoacronycine (**12**). In the ^{1}H nmr spectrum of this compound, four coupled aromatic signals (δ_H 7.26, 7.44, 7.69 and 8.47) and a *N*-Me signal (δ_H 3.75) were quite similar to those of **11** and **12**[6], and a set of geminal methyl signals appeared at δ_H 1.29 (3H, s) and 1.47 (3H, s). However, no *O*-Me signal was observed. Instead, a methyl signal was observed at δ_H 1.49 (3H, d, J = 7 Hz), which was coupled with the resonance at δ_H 3.12 (1H, ddq, J = 7, 10, 7 Hz, H-1'). Furthermore, this signal at δ_H 3.12 was coupled with the methylene signals at δ_H 1.68 (1H, dd, J = 10, 14 Hz) and 2.05 (1H, dd, J = 7, 14 Hz). From these results, the alternative structures (**13**) and (**14**) were deduced. NOe experiments on this compound showed enhancement at δ_H 6.28 (1H, s) and 7.44 (1H, d) on irradiation of the *N*-Me signal (δ_H 3.75). Thus, the linear structure (**13**) was concluded for this product. The co-occurrence of **9** and **13** in the reaction products suggested that an additional methyl moiety of **13** at $C_{1'}$ position is derived from $C_{1'}$ or $C_{2'}$-CH_2 of compound (**5**).



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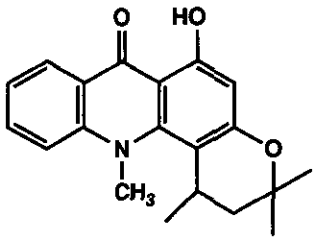

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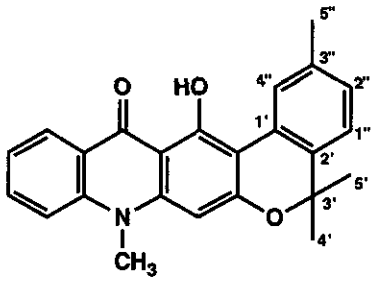
The last isolate (**15**) was obtained as orange needles and this compound was also estimated to be an acridone alkaloid. In the EIms of this compound, a molecular ion at *m/z* 371 was observed and its molecular formula ($C_{24}H_{21}NO_3$) was elucidated by hreims. On the other hand, in the ^{13}C nmr spectrum, 18 aromatic carbon signals were observed, together with signals attributed to a methyl resonance (δ_C 21.7) attached to benzene ring, a geminal methyl (δ_C 27.8, 2 x C), a *N*-Me signal (δ_C 34.0), a quaternary signal (δ_C 79.0) and a carbonyl resonance at δ_C 181.2. The higher field chemical shift of the *N*-Me signal in the ^{13}C NMR spectrum of **15** indicated that this acridone alkaloid possessed a linear skeleton.[5] In the ^1H nmr of this product, a singlet aromatic signal at δ_H 8.59, a set of coupled aromatic doublets at δ_H 7.08 (1H, d, J = 8 Hz) and 7.15 (1H, d, J = 8 Hz) and a methyl signal attached to an aromatic ring were observed, in addition to a set of four aromatic signals (δ_H 7.29, 7.47, 7.71, 8.49), a singlet aromatic resonance at δ_H 6.47, a geminal methyl resonance (δ_H 1.66 (6H, s)) and a hydrogen bonded OH signal at δ_H 13.68. So, the existence of an additional benzene ring with 1,2,4-substitution was estimated and the newly appeared aromatic signal at δ_H 8.59 (1H, s) was assigned to H-4" by comparison with the reported data[8] for (+)-isopiline and the structure of this compound was concluded to be **15**. ^1H and ^{13}C NMR assignments of **15** have been achieved by HMBC spectra. This compound possessed an additional C_5 unit ($C_{1"}$ - $C_{5"}$) compared with **5** and is considered to be derived from the dimer with C-C bond between the prenyl moieties of two acridone units as reported previously.[5,9]



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Compounds (**6**, **9**, **13** and **15**) have not been reported previously and none of these compounds is present as a contaminat in the original acronycine.

EXPERIMENTAL

General Experimental Procedures - The ir spectra were recorded on a Jasco A-100S infrared spectrophotometer and the uv spectra were obtained on a Hitachi U-3200 spectrophotometer. ^1H- and ^{13}C-nmr spectral analysis were performed on a 300 MHz FT-nmr Hitachi R-3000 or a 500 MHz FT-nmr JEOL JNM-GX500 in $CDCl_3$ or CD_3OD using TMS (tetramethylsilane) as internal standard. Mass spectra were recorded with a JEOL JMS-AX-

500 or JMS-DX303 mass spectrometer. Wako gel C-200 (Wako Pure Chemical Industries Ltd.) was used for column chromatography. Silica gel 60 F_{254} (Merck) plates were used for tlc analysis and ptlc. Spots on tlc were detected by uv lamp (254 nm) and/or by spraying with 2% $FeCl_3$/MeOH reagent.

Preparation of HCl Salt of Acronycine (1) - The HCl salt of acronycine (**1**) was prepared as reported previously.[7]

Treatment of the HCl Salt of Acronycine (1) - Acronycine·HCl (16.72 g) was heated at 250°C on a mantel heater for 2.5 h to afford a dark brown reaction product.

Isolation and Purification of the Compounds - The reaction mixture was extracted with $CHCl_3$ to yield an extract (9.72 g) and a residue (5.59 g).

Part of the $CHCl_3$ extract (8.76 g) was chromatographed over silica gel to afford frs I (0.097 g), II (0.174 g), III (2.054 g), IV (0.601 g), V (5.337 g) and VI (1.367 g). Fr III was reapplied to silica gel column chromatography and the column was eluted with $CHCl_3$ to afford dihydronorisoacronycine (**5**, 1089.0 mg, 7.2%), compound (**9**) (1.3 mg, 0.009%) and compound (**15**) (301.2 mg, 2.0%). Part of fr. IV (10.5 mg) was acetylated with Ac_2O/pyridine to afford **7** (2.3 mg). The yield of **6** (0.75%) was calculated by the yield of **7** and part of fr. II (20.0 mg) was purified by ptlc (C_6H_6-$CHCl_3$ = 1:1) to afford compound **13** (0.45%). On the other hand, compounds (**2**) (0.009%), (**3**) (0.016%) and (**4**) (0.64%) were isolated from frs III (**2** and **3**) and IV (**4**), respectively.

Identification of Noracronycine (2), Dihydronoracronycine (3), 1,3-Dihydroxy-10-Methylacridone (4), and *Dihydronorisoacronycine* (5) - *Identification* of noracronycine (**2**), dihydronoracronycine (**3**), 1,3-dihydroxy-10-methylacridone (**4**) and dihydronorisoacronycine (**5**) was conducted by direct comparison (mtlc) with the *authentic samples prepared previously.*[6]

Physicochemical Properties of Compounds (7, 9, 13 and 15) - Compound (**7**) was obtained as a yellow powder. mp 214 - 219°C. Uv, λ_{max} (MeOH) 264, 310, 408 nm; ir, ν_{max} ($CHCl_3$) 3000, 1770, 1626, 1610, 1570, 1508, 1378, 1320, 1290, 1195, 1102 cm^{-1}; EIms, *m/z* 297 (M^+), 255, 241, 226, 212, 183, 154; HREIms, *m/z* obsd 297.1022, calcd for $C_{17}H_{15}NO_4$ 297.1001; ^1H nmr ($CDCl_3$) δ 2.12 (3H, s, 2-Me), 2.39 (3H, s, 3-OAc), 3.81 (3H, s, N-Me), 6.62 (1H, s, H-4), 7.31 (1H, dd, *J* = 8, 8 Hz, H-7), 7.50 (1H, d, *J* = 9 Hz, H-5), 7.75 (1H, ddd, *J* = 1.5, 8, 9 Hz, H-6), 8.49 (1H, dd, *J* = 1.5, 8 Hz), 14.98 (1H, s, 1-OH). Anal. Calcd for $C_{17}H_{15}NO_4$: C, 68.68; H, 5.07; N, 4.71. Found: C, 68.97; H, 5.42; N, 4.93.

Compound (**9**) was obtained as a yellow powder. mp 242 - 248°C. Uv, λ_{max} (MeOH) 274, 330, 392 nm; ir, ν_{max} ($CHCl_3$) 2870, 1650, 1600, 1500, 1460, 1328, 1108, 1070 cm^{-1}; EIms, *m/z* 295 (M^+), 280, 266, 254, 149, 83, 77; HREIms, *m/z* obsd 295.1211, calcd for $C_{18}H_{17}NO_3$ 295.1209; ^1H nmr ($CDCl_3$) δ 1.55 and 1.56 (each 3H, s, gem-Me), 3.07 (2H, s, H_2-1'), 3.80 (3H, s, N-Me), 6.28 (1H, s, H-4), 7.30 (1H, dd, *J* = 8, 8 Hz, H-7), 7.49 (1H, d, *J* = 9 Hz, H-5), 7.71 (1H, ddd, *J* = 1.5, 8, 9 Hz, H-6), 8.48 (1H, dd, *J* = 1.5, 8 Hz, H-8), 15.09 (1H, s, 1-OH).

Compound (**13**) was obtained as an orange oil. Uv, λ_{max} (MeOH) 249, 275, 331, 404 nm; ir, ν_{max} ($CHCl_3$) 3000, 2880, 1640, 1602, 1570, 1465, 1340, 1283, 1200, 1138, 930, 895 cm^{-1}; EIms, *m/z* 323 (M^+), 308, 266, 254, 226, 210, 167, 147, 140; HREIms, *m/z* obsd 323.1542, calcd for $C_{20}H_{21}NO_3$ 323.1521; ^1H nmr ($CDCl_3$) δ 1.29 and 1.47 (each 3H, s, gem-Me), 1.49 (3H, d, *J* = 7 Hz, 1'-Me), 1.68 (1H, dd, *J* = 10, 14 Hz, H-2'), 2.05 (1H, dd, *J* = 7, 14 Hz, H-2'), 3.12 (1H, ddq, *J* = 7, 10, 7 Hz, H-1'), 3.75 (3H, s, N-Me), 6.28 (1H, s, H-4), 7.26 (1H, dd, *J* = 8, 8 Hz, H-7), 7.44 (1H, d, *J* = 9 Hz, H-5), 7.69 (1H, ddd, *J* = 1.1, 8, 9 Hz, H-6), 8.47 (1H, dd, *J* = 1.1, 8 Hz, H-8), 15.35 (1H, s, 1-OH). Compound (**15**) was obtained as orange needles from $CHCl_3$-MeOH. mp 218 - 221°C. Uv, λ_{max} (MeOH) 246, 307, 407 nm; ir, ν_{max} ($CHCl_3$) 2870, 1650, 1500, 1460, 1328, 1108, 1070 cm^{-1}; EIms, *m/z* 371 (M^+), 356, 341, 185, 178, 170; HREIms, *m/z* obsd 371.1531, calcd for $C_{24}H_{21}NO_3$ 371.1521; ^1H nmr ($CDCl_3$) δ 1.66 (6H, s, gem-Me), 2.44 (3H, s, 3"-Me), 3.81 (3H, s, N-Me), 6.47 (1H, s, H-4), 7.08 (1H, d, *J* = 8 Hz, H-1"), 7.15 (1H, d, *J* = 8 Hz, H-2"), 7.29 (1H, dd, *J* = 7, 8 Hz, H-7), 7.47 (1H, d, *J* = 7 Hz, H-5), 7.71 (1H, ddd, *J* = 1.5, 7, 8 Hz, H-6), 8.49 (1H, dd, *J* = 1.5, 8 Hz, H-8), 8.59 (1H, s, H-4"), 13.68 (1H, s, 1-OH); ^{13}C nmr ($CDCl_3$) δ 21.7 (C-5"), 27.8 (2 x C, gem-Me), 34.0 (N_{10}-Me), 79.0 (C-3'), 92.7 (C-4), 103.8 (C-9a), 106.0 (C-2), 114.5 (C-5), 121.0 (C-8a), 121.6 (C-7), 122.3 (C-1"), 126.9 (C-8), 126.9 (C-1'), 127.0 (C-4"), 127.6 (C-2"), 134.1 (C-6), 135.5 (C-3"), 137.4 (C-2'), 142.1 (C-10a), 144.0 (C-4a), 160.9 (C-3), 163.3 (C-1), 181.2 (C-9). Anal. Calcd for $C_{24}H_{21}NO_3$: C, 77.62; H, 5.68; N, 3.77. Found: C, 77.70; H, 5.67; N, 3.74.

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