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Lucibufagins. 2. Esters of 12-Oxo-2β,5β,11α-trihydroxybufalin, the Major Defensive Steroids of the Firefly *Photinus pyralis* (Coleoptera: Lampyridae)[1a]

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Abstract: Fireflies of the species *Photinus pyralis* (Coleoptera: Lampyridae) are shown to contain a group of closely related steroidal pyrones, named lucibufagins, which serve a defensive role. The five major steroids from this insect are characterized as esters (**5**, **6**, **7**, **11**, **12**) of 12-oxo-2β,5β,11α-trihydroxybufalin (**9**), chiefly on the basis of detailed spectral analyses. Selective proton decoupling ^{13}C NMR experiments played a key role in making these structural assignments. These insect-derived steroids are the first bufadienolides to be isolated and characterized from an invertebrate source.

Introduction

The remarkable ability of fireflies (sometimes also called lightning bugs or glowworms) to produce light signals has been celebrated by poets[2] and musicians.[3] Biologists,[4] biochemists,[5] and organic chemists[6] have studied the origin and meaning of these beetles' signals in depth. We have become interested in the chemical defenses of fireflies, and have recently reported that two locally available species, *Photinus ignitus* and *P. marginellus*, contain mixtures of at least three bufadienolides, for which we proposed the generic name lucibufagins.[7] These steroids were shown to render the fireflies distasteful to birds.[7] We now report the complete structures of five lucibufagins isolated from *P. pyralis*.[8] These insect steroids are the first insect-derived bufadienolides to be fully characterized. Data on their deterrent effect on birds and ants will be described elsewhere.

Results and Discussion

Employing the extraction procedure previously described,[7] ~2.8 g of crude steroid was isolated from ~28 000 freeze-dried, lanternectomized animals (*P. pyralis*).[8] This extract was further fractionated by column chromatography, which afforded five compounds (initially designated as A, B, C, D, and E in order of increasing retention volume) in the ratio of 2:1:20:8:3. The major component, compound C, crystallized spontaneously, providing over 1 g of pure material. We focused our first efforts on determining the structure of this compound.

The infrared spectrum of C contains significant bands at 3550 and 3430 (OH), 1750–1700 (overlapping carbonyls), and 1635 and 1535 cm^{-} (C=C), while its ultraviolet spectrum has a single maximum at 298 nm (ε 5500). The ultraviolet absorption, together with the observation of a set of three interacting protons in the low-field region of the ^1H NMR spectrum of C (Figure 1), indicates the presence of a conjugated system. The chemical shifts and coupling constants of the three low-field protons agree closely with those reported for 5-substituted 2-pyrones[9] and, upon consideration of all of the spectral data, the presence of this pyrone moiety in C is clear.[10,11] The chemical ionization mass spectrum (CIMS) of C establishes a molecular weight of 532, leaving 437 amu to be accounted for by the single substituent on the pyrone ring.

Our initial interpretation of the ^1H NMR spectrum led to the hypothesis that C contains two quaternary methyl groups, two secondary acetoxy groups, and a tertiary and two secondary hydroxyl groups. The ^{13}C NMR spectrum shows 28 resonances and confirms the presence of two acetates (δ 20.5, 20.7, 168.8, and 169.2). This accounts for two of a total of five sp^3 carbons bearing an oxygen substituent. In addition, a set of five resonances (δ 114.9, 120.9, 147.5, 149.8, and 162.5) agree well with expectations for the α-pyrone unit.[12] Finally, a signal observed at δ 212.8 revealed the presence of a ketonic carbonyl group. With 28 carbons directly observed and 10 oxygens implied from the ^{13}C NMR spectrum of C, the molecular formula $C_{28}H_{36}O_{10}$ (mol wt 532) is required by its mass spectrum. Aside from the nine carbon resonances of the pyrone and acetate units, the remaining 19 signals present a pattern which suggests that C contains a saturated steroid nucleus, a conclusion consistent with the molecular formula and with the presence of two upfield methyl singlets in its ^1H NMR spectrum.

Steroidal pyrones, previously isolated only from certain toads and a small group of plants,[13] often possess desirable cardiotonic properties. This class of steroids, the bufadienolides, is characterized by a cis-anti-trans-syn-cis steroid skeleton bearing 3β- and 14β-oxygen substituents and a 17β-pyrone substituent. We assumed (and later confirmed) the presence of these same features in compound C, placing an acetoxy group at the C-3 position (vide infra) to derive the part structure **1**.

The electron impact mass spectrum (EIMS) of C, although dominated by cleavage of the acetyl groups (*m/e* 43), does contain useful fragments of higher molecular weight. In addition to the weak molecular ion (*m/e* 532), diagnostic peaks were observed at *m/e* 123, 135, 136, 191, 201, and 204. The three lower molecular weight fragments in this group are characteristic of bufadienolides which are devoid of oxygen substituents at C-15, -16 and -17.[14] The three remaining ions, also prominent in the mass spectrum of arenobufagin (**2**), have

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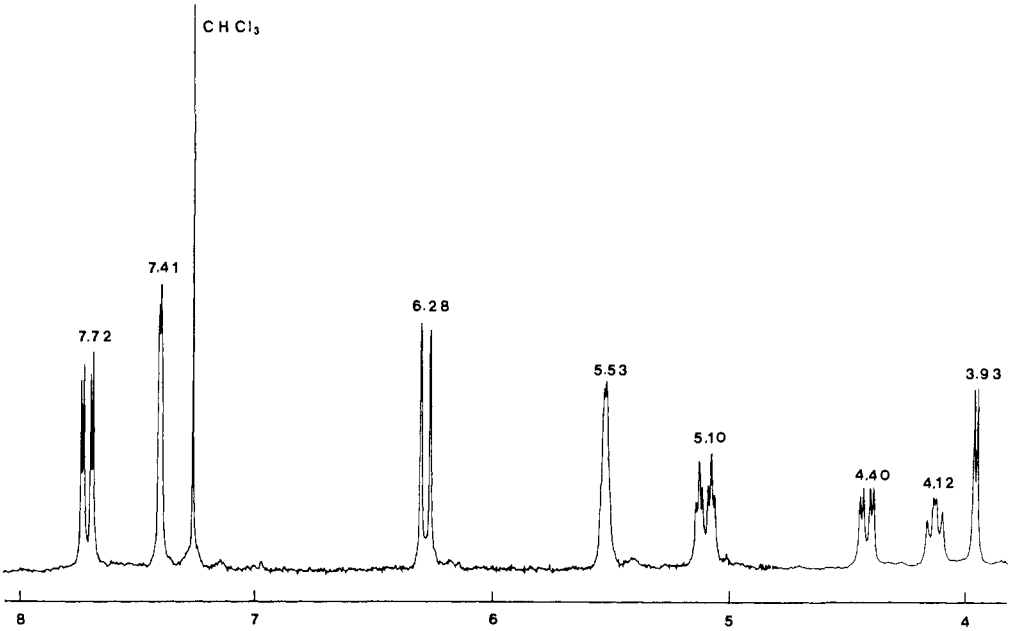
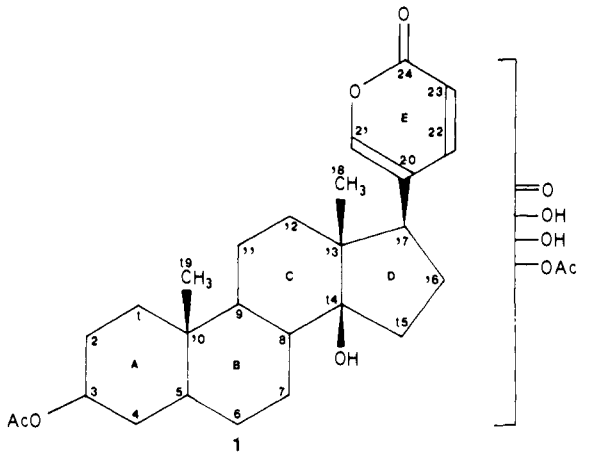
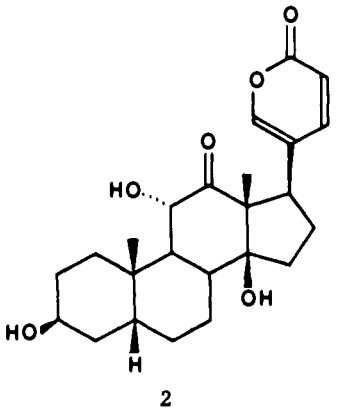


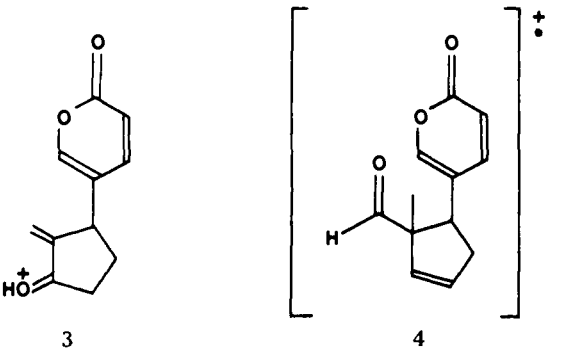
Figure 1. Low-field region of the 250-MHz ^1H NMR spectrum of compound C (**5**) in $CDCl_3$.





been assigned structures which include the pyrone ring, the 14-hydroxylated D ring, and the C-12 carbonyl group on the basis of high resolution mass spectrometry.[14] The mass spectrum of C therefore suggests the presence of a carbonyl group at C-12 as well as the usual C-14 hydroxyl group.

Treatment of C with methanolic ammonia gave a deacetylated steroid whose mass spectrum was instructive. A molecular ion at *m/e* 448 (11%) was detected, as well as the characteristic peaks at *m/e* 123, 135, 136, 191, 201, and 204, previously observed in the mass spectrum of C itself. High resolution mass measurements established the anticipated molecular formula $C_{24}H_{32}O_8$ for this deacetylated product, and confirmed the compositions of four important fragment ions (Table I). Of these, the *m/e* 191 and 204 fragments, previously characterized as **3** and **4** by Pettit et al.,[14] are particularly



important because they provide direct support for the presence of C-12 carbonyl and C-14 hydroxyl functions, and for the absence of oxygen substituents at C-15, -16 and -17.

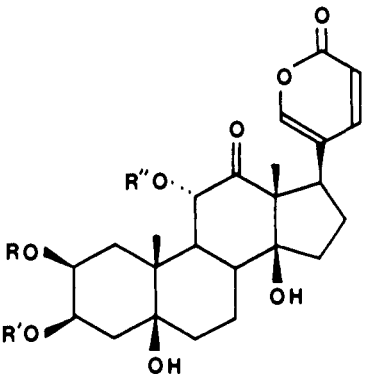
In order to establish the positions of the remaining oxygen substituents, it was necessary to resolve an apparent discrepancy between the ^1H and the ^{13}C NMR spectra. Off-resonance decoupling experiments established that of the five sp^3 carbon atoms bonded to oxygen, *two* bear no protons (δ 84.1 (s) and 73.1 (s)), and *three* bear a single proton (δ 72.8 (d), 70.0 (d), and 67.8 (d)). However, our initial interpretation of the ^1H NMR spectrum had suggested that *four* oxygen substituents were secondary and only *one* tertiary. In order to resolve this conflict, we conducted selective proton decoupling experiments,

Table I. High Resolution Mass Spectral Data for Deacetylated Compound C

m/e	formula	measd mass	calcd mass
448	$C_{24}H_{32}O_8$	448.2075	448.2097
430	$C_{24}H_{30}O_7$	430.1978	430.1991
412	$C_{24}H_{28}O_6$	412.1901	412.1886
204	$C_{12}H_{12}O_3$	204.0770	204.0786
191	$C_{11}H_{11}O_3$	191.0730	191.0708
191	$C_{12}H_{15}O_2$	191.1092	191.1072

carefully controlled to decouple single protons. These experiments revealed that the protons found at δ 5.5, 5.1 (2 C*H*OAc), and 4.4 (C*H*OH) are bonded to the carbons appearing at δ 70.0, 67.8, and 72.8 ppm, respectively. However, the remaining downfield proton (δ 4.1) was found to be joined to a carbon atom which absorbs at δ 40.4 (d), a position too far upfield to represent an oxygen-bearing carbon. Thus it can be concluded that compound C contains two secondary acetoxy functions, one secondary hydroxyl group, and therefore two tertiary hydroxyls (one of which is already known to be at C-14). The nature of the methine proton which appears at δ 4.1 (d of d, J = 9, 6 Hz) remains to be explained.

A more detailed examination of the ^{1}H NMR spectral data allows determination of the positions of the remaining functional groups, and leads ultimately to the assignment of structure **5** to C. Of the remaining protons geminal to oxygen, one appears at δ 4.40 as a doublet of doublets (J = 10, 3 Hz) which simplifies to a doublet (J = 10 Hz) upon addition of D_2O. This simple splitting pattern places this hydroxyl group at C-11; the diaxial arrangement of the C-11 and C-9 protons is apparent from the large coupling constant, and necessitates the equatorial (α) placement of the hydroxyl group. With this result in hand, it becomes possible to assign the position of the remaining (tertiary) hydroxyl group. Of the four possible tertiary sites (C-5, C-8, C-9, and C-17), two (C-9 and C-17) already have been eliminated on the basis of ^{1}H NMR and mass spectral data. Introduction of an 8β- or 5α-hydroxyl group is known to result in dramatic deshielding of the angular methyl groups[15] and is not compatible with the observed chemical shifts. Thus the ^{1}H NMR spectrum appears most consistent with the 5β placement of this tertiary hydroxyl group.

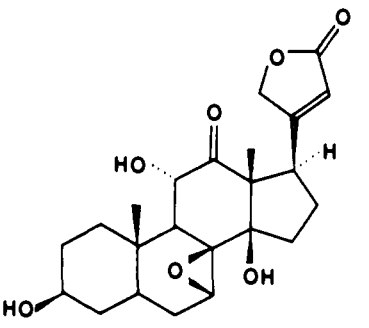


	R	R'	R''
5	CH_3CO	CH_3CO	H
6	CH_3CO	H	H
7	H	CH_3CO	H
8	CH_3CO	CH_3CO	CH_3CO
9	H	H	H
10	CH_3CO	$(CH_3)_2CHCO$	$(CH_3)_2CHCO$
11	CH_3CO	$(CH_3)_2CHCO$	H
12	CH_3CO	CH_3CH_2CO	H
16	CH_3CO	CH_3CH_2CO	CH_3CH_2CO

The two secondary acetoxy groups can be located by inspection of the coupling patterns and chemical shifts of their geminal protons. The doublet observed at δ 5.53 (Figure 1) is too narrow to include a diaxial coupling. This signal is most easily accounted for by the assignment of one acetoxy group to the 3β position, an oxygenation pattern almost universally observed in bufadienolides.[16] It is interesting to note that in eight known 3β-acetoxybufadienolides the 3α proton appears at δ 5.09 ± 0.02.[15] In bufadienolides which also bear a 5β-hydroxyl substituent, intramolecular hydrogen bonding has been invoked to explain a marked deshielding of the 3α proton (δ 5.28 ± 0.01),[15] as well as a more modest deshielding of the acetate methyl (δ 2.09 vs. 2.04).[15] In compound C, one acetate methyl is observed at δ 2.10, in accord with expectations for this type of interaction. The 3α proton, however, appears even further downfield than expected (δ 5.55). This implies that still another electron-withdrawing group is nearby; the only possibility is the one remaining acetoxy group. The proton geminal to this final acetoxy group appears at δ 5.10 as a doublet of triplets (J = 12, 4, 3 Hz), suggesting one diaxial coupling and two axial-equatorial couplings. Of the remaining positions on the A and B rings, this pattern can be achieved only by placement of the acetoxy group at the 2β position, leading unambiguously to structure **5**. While all the functional groups are well accommodated for in this structure, the origin of the ^{1}H NMR absorption at δ 4.1 was not immediately apparent. However, the spectral studies of arenobufagin (**2**) described below resolved this problem.

In the ^{1}H NMR spectrum of **2** there are signals easily attributable to the 11α proton (δ 4.35, d of d, J = 10, 3 Hz) and to the 11-hydroxyl proton (δ 3.82, d, J = 3 Hz, exchanges with D_2O). These signals correspond closely in chemical shift and coupling pattern to the analogous protons of **5**. Furthermore, using the observed chemical shifts of arenobufagin's angular methyl groups as base values and employing the empirical rules of Gsell and Tamm[15] to incorporate the effects of acetylation of C-3 and hydroxylation at C-5, it is possible to calculate the expected chemical shifts for the angular methyl groups of **5**. The calculated (C-18, δ 0.95; C-19, δ 1.20) and the observed values (C-18, δ 0.89; C-19, δ 1.20) agree well, providing additional support for the position and stereochemistry of the function groups.[17]

The ^{1}H NMR spectrum of **2** contains two overlapping absorptions at δ 4.10. Acetylation results in a downfield shift of ~1 ppm for one of these signals, presumably the 3α proton, while the second signal, whose splitting pattern can now be seen (d of d, J = 9, 6 Hz), is unaffected. This absorption can be attributed only to the C-17 proton, strongly deshielded by proximity to the C-12 carbonyl group. Similar deshielding of a 17α proton in a 12-ketocardenolide has been reported in the case of sarverogenin (**13**).[18] The analogous δ 4.1 signal in the



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^{1}H NMR spectrum of **5** can therefore be assigned with confidence to the 17α proton in this C/D cis-fused 12-ketobufadienolide.

To help assign the ^{13}C resonances in **5**, the ^{13}C NMR spectrum of **2** was recorded. Many of the resonances could be assigned by comparison with pyrone[12] and cardenolide[19] models. Close agreement is observed between the spectra of **2** and **5**, and thus many assignments can be made (Figure 2). The signals attributed to C-11–C-24, except for C-19, are virtually identical; especially significant are the signals ascribed to C-13 (deshielded to an unusual extent by the adjacent carbonyl) and C-14. The signal attributed to C-3 is observed downfield by 3.6 ppm in **5** vs. **2**, a shift compatible with acetylation of this position.[19] Introduction of the C-5 hydroxyl group has significantly shifted the easily recognized C-10 and C-19 resonances. The observed Δδ values agree nicely with

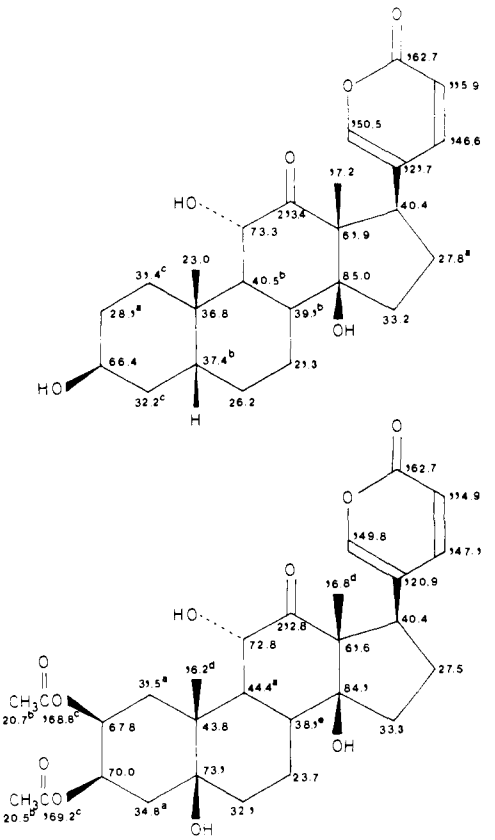

Figure 2. ^{13}C NMR assignments for **2** and **5**. Resonances with the same superscripts may be interchanged.

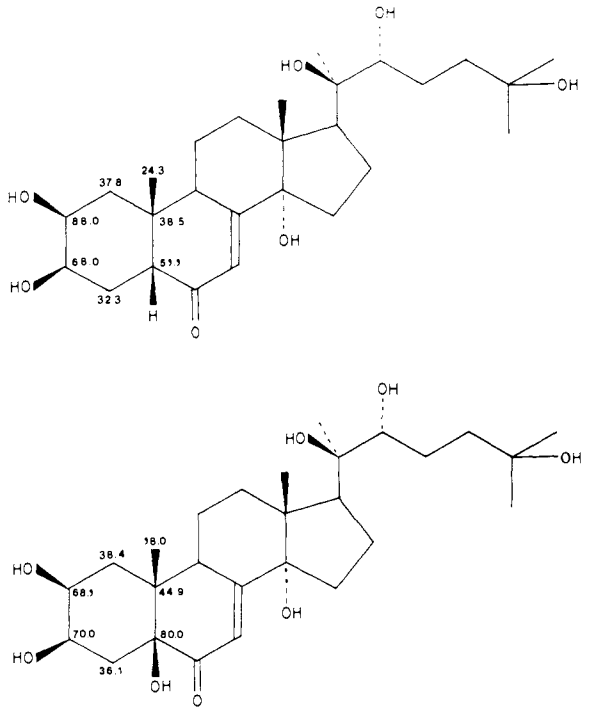

Figure 3. Partial ^{13}C NMR assignments for **14** and **15**. Note $\Delta\delta$ values of +6.4 and −6.3 at C-10 and C-19, respectively, resulting from the introduction of a 5β-OH.

those recently reported[20] for the two ecdysones **14** and **15** (Figure 3).

On the basis of all of these considerations, the major lucibufagin of *P. pyralis* is seen to be 12-oxo-2β,3β-di-*O*-acetyl-5β,11α-dihydroxybufalin (**5**). With this structure determined, the structures of the remaining four lucibufagins from this insect were rapidly established. The electron impact mass spectra of both D and E show weak molecular ions at *m/e* 490 (D, 3%; E, 3%), as well as fragments characteristic of the pyrone ring (D, 123 (32), 135 (23), 136 (12); E, 123 (29), 135 (25), 136 (11)), and those which imply C-12 carbonyl and C-14 hydroxyl groups (D, 191 (42), 201 (19), 204 (43); E, 191 (33), 201 (17), 204 (32)). These data suggest that D and E are isomeric monoacetates, closely related to **5** itself.

The NMR data confirm this hypothesis and allow placement of the single acetyl group in each compound. For D, the ^{13}C NMR spectrum shows 26 peaks; 22 of these occur within 0.6 ppm of the analogous signals in the spectrum of **5**. The ^1H NMR spectrum of D reveals a single acetyl methyl group and only one proton geminal to an acetoxy group (δ 5.15, d of t, J = 3, 3, 12 Hz). The splitting pattern of this proton is identical with that observed for the 2α proton of **5**, indicating that D is the monoacetate **6**. For E, the ^{13}C NMR spectrum also shows 26 resonances; 23 of these peaks are within 0.3 ppm of the analogous signals found in the spectrum of **5**. The ^1H NMR spectrum of E has a single acetyl methyl group absorption and only one proton geminal to an acetoxy group (δ 5.4). This proton has a pattern identical with that corresponding to the 3α proton of **5**. These data indicate that E is the monoacetate **7**. Treatment of **5**, **6**, and **7** with acetic anhydride in pyridine gives the same triacetate, **8**.

Spectral data indicated clearly that compounds A and B are also esters of 12-oxo-2β,5β,11α-trihydroxybufalin (**9**) obtained earlier by ammonolysis of C (**5**) (vide supra). The mass spectrum of A shows a molecular weight of 560, along with the now familiar series of fragment ions which are diagnostic for the pyrone ring plus the C-12 carbonyl and C-14 hydroxyl functions. In common with the mass spectra of **5**, **6**, and **7**, the base peak in the mass spectrum of A (*m/e* 43) results from cleavage of an acetyl moiety, but a new peak at *m/e* 71 (43%) implies the presence of a larger acyl group as well. This is confirmed by the ^{13}C NMR spectrum of A, which exhibits 30 signals, 26 of which find close counterparts in the spectrum of **5** and four of which can be ascribed to an isobutyrate residue in place of one of the two acetate functions in **5**. The low-field portion of the ^1H NMR spectrum of A is virtually identical with that of **5**, indicating acylation of both the 2β- and 3β-hydroxy groups, while the upfield region contains two new doublets, as expected for the methyl groups of an isobutyrate ester. In order to determine which acyl group was at C-2 and which at C-3, samples of the monoacetates **6** and **7** were each treated with isobutyryl chloride in pyridine, and the course of the acylation was followed by LC. In the case of **6**, the initial product had a retention time (R_t) identical with that of A, and this was further esterified to yield a bisisobutyrate (**10**) of slightly longer R_t. In contrast, the initial product from **7** did not agree in R_t with A, and a new bis ester was produced. Treatment of A itself with isobutyryl chloride afforded a bisisobutyrate (**10**) indistinguishable from that obtained from **6**. A can therefore be assigned structure **11**. In a completely analogous fashion, B was shown to be the 2-acetate 3-propionate (**12**) (see the Experimental Section for details).

These five steroids (**5**, **6**, **7**, **11**, and **12**) are the first bufadienolides to be isolated and characterized from an arthropod source. In view of the known biological properties of the bufadienolide family, and our preliminary bioassays[7] which revealed that these steroids render fireflies distasteful to birds, it seems clear that these compounds have a defensive value to the fireflies. The other biological properties of these bufadienolides, as well as their distribution among the Lampyridae and related animals, are subjects under active investigation.

Experimental Section

Melting points were recorded on a Thomas-Hoover melting point

apparatus and are uncorrected. Ultraviolet spectra were obtained on a Cary 14 spectrophotometer and infrared spectra (in $CHCl_3$) were recorded with a Perkin-Elmer 257 grating infrared instrument. The 90-MHz ^1H NMR spectra were obtained on a Varian EM-390 instrument while the 250-MHz spectrum of **5** was recorded at the NIH NMR Facility for Biomedical Studies, Mellon Institute, Pittsburgh. Deuteriochloroform was used as solvent unless otherwise noted and chemical shifts are reported in parts per million downfield from $(CH_3)_4Si$. Routine ^{13}C NMR spectra were obtained on a Varian CFT-20 spectrometer with the following parameters representing a typical set of conditions: spectral width, 4000 Hz; acquisition time, 1.023 s; pulse width, 10 μs; data points, 8192. Chemical shifts are reported in parts per million downfield from $(CH_3)_4Si$ using the chloroform resonance as the internal standard (76.89). Spectra were recorded using 10% methanol in deuteriochloroform as solvent. The selective proton decoupling experiments were conducted on a Bruker FX-90 instrument. Low resolution mass spectra were recorded with a Finnigan Model 3300 instrument coupled to a Systems Industries Model 150 computer; only selected ions are reported here. High resolution mass spectra were obtained on an AEI MS-902 mass spectrometer coupled to a VG Data System 2020 computer. The LC analyses were conducted with a Waters Model 6000 solvent delivery system and a Waters Model 440 ultraviolet detector. Routine analyses were conducted on a μ Porasil column using 50% hexane in tetrahydrofuran as the solvent system.

Isolation of 5, 6, 7, 11, and 12. Freeze-dried and lanternectomized *P. pyralis* (28 000 animals, ~280 g) were suspended in dichloromethane (3 L) and allowed to stand at 0 °C for 2 days.[21] The mixture was then filtered and the resulting solution concentrated in vacuo to afford an oily residue. After this residue was washed with hexane, ~2.8 g of crude steroid remained as an amorphous solid. Fractionation by column chromatography on silica gel (gradient elution, 100% hexane to 100% tetrahydrofuran) afforded A (**11**, 0.14 g), B (**12**, 0.07 g), C (**5**, 1.41 g), D (**6**, 0.55 g), and E (**7**, 0.21 g).

12-Oxo-2β,3β-di-*O*-acetyl-5β,11α-dihydroxybufalin (5). A sample of **5**, which had spontaneously crystallized after chromatography, recrystallized from absolute ethanol as transparent needles which became opaque at 148 °C, effervescent at 158–160 °C, and melted with decomposition at 185–190 °C: $[\alpha]_D^{EtOH}$ +35.5° (*c* 1.3); UV $\lambda_{max}^{H_2O}$ 298 nm (ε 5500); IR ($CHCl_3$), 3550, 3430, 1750–1700, 1635, and 1535 cm^{-1}; ^1H NMR δ 0.93 (3, s), 1.26 (3, s), 1.2–2.1 (14, m), 1.98 (3, s), 2.14 (3, s), 2.23 (1, d of d, *J* = 15.9, 3.1 Hz), 2.50 (1, d of d, *J* = 13.6, 3.2 Hz), 3.93 (1, d, *J* = 3.7 Hz, exchanges with D_2O), 4.12 (1, d of d, *J* = 6.6, 2.9 Hz), 4.40 (1, d of d, *J* = 10.7, 3.5 Hz, addition of D_2O simplifies to a d, *J* = 10.7 Hz), 5.10 (1, d of t, *J* = 12.6, 3.9, 3.4 Hz), 5.53 (1, br d, *J* = 2.2 Hz), 6.28 (1, d, *J* = 9.7 Hz), 7.41 (1, d, *J* = 1.7 Hz), 7.72 (1, d of d, *J* = 9.7, 2.5 Hz); for ^{13}C NMR, see Figure 2; EIMS (70 eV) *m/e* (rel intensity) 532 (M^+, 2) 514 ($M^+ - H_2O$, 5), 496 ($M^+ - 2H_2O$, 2), 472 (1), 454 (2), 436 (5), 412 (6), 394 (13), 376 (9) 366 (6), 354 (5), 221 (25), 204 (36), 201 (11), 191 (27), 175 (19), 136 (8), 135 (16), 123 (18), 43 (100); CIMS (isobutane) *m/e* (rel intensity) 533 ($M^+ + 1$, 43).

12-Oxo-2β-*O*-acetyl-5β,11α-dihydroxybufalin (6). A sample of **6** was crystallized from chloroform: mp 186–187 °C; $[\alpha]_D^{EtOH}$ +36.4° (*c* 1.4); ^1H NMR δ 0.92 (3, s), 1.21 (3, s), 1.2–2.6 (17, m), 2.10 (3, s), 3.90 (1, br, exchanges with D_2O), 4.15 (1, d of d, *J* = 9,6 Hz), 4.25 (1, br), 4.40 (1, br d, addition of D_2O simplifies to d, *J* = 12 Hz), 5.08 (1, d of t, *J* = 12, 3, 3 Hz), 6.28 (1, d, *J* = 10 Hz), 7.43 (1, d, *J* = 2 Hz), 7.75 (1, d of d, *J* = 10, 2 Hz); ^{13}C NMR δ 212.6 (s), 168.8 (s), 162.5 (s), 149.8 (d), 147.1 (d), 121.0 (s), 114.9 (d), 84.3 (s), 74.1 (s), 73.1 (d), 70.9 (d), 68.3 (d), 61.7 (s), 44.4 (d), 43.9 (s), 40.5 (d), 38.3 (d), 35.8 (t), 33.7 (t), 32.1 (t), 30.9 (t), 27.6 (t), 23.2 (t), 20.8 (q), 16.9 (q), 16.1 (q); EIMS (70 eV) *m/e* (rel intensity) 490 (M^+, 3), 472 ($M^+ - H_2O$, 8), 454 ($M^+ - 2H_2O$, 9), 394 (24), 376 (12), 366 (10), 354 (6), 221 (23), 214 (15), 204 (43), 201 (19), 191 (42), 175 (36), 136 (12), 135 (25), 123 (32), 43 (100).

12-Oxo-3β-*O*-acetyl-2β,5β,11α-trihydroxybufalin (7). Compound **7** was obtained as a glass which resisted crystallization, although it appeared homogeneous on the basis of LC analysis: $[\alpha]_D^{EtOH}$ +20.4° (*c* 1.08); ^1H NMR δ 0.92 (3, s), 1.21 (3, s), 1.30–2.7 (17, m), 2.15 (3, s), 3.2 (1, br, exchanges with D_2O), 3.9–4.3 (2, m), 4.42 (1, d, *J* = 12 Hz), 5.40 (1, br d, *J* = 3 Hz), 6.30 (1, d, *J* = 9 Hz), 7.45 (1, d, *J* = 2 Hz), 7.76 (1, d of d, *J* = 9,2 Hz); ^{13}C NMR δ 212.9 (s), 168.5 (s), 162.5 (s), 149.8 (d), 147.1 (d), 120.9 (s), 115.0 (d), 84.2 (s), 73.1 (s), 73.0 (d), 73.0 (d), 65.2 (d), 61.7 (s), 44.5 (d), 43.6 (s), 40.5 (d), 38.3 (d), 35.0 (t), 35.0 (t), 33.3 (t), 32.1 (t), 27.6 (t), 23.4 (t), 20.9 (q), 16.9 (q), 16.3 (q); EIMS (70 eV) *m/e* (rel intensity) 490 (M^+, 3), 472 ($M^+ - H_2O$, 5), 454 ($M^+ - 2H_2O$, 6), 394 (18), 376 (10), 366 (8), 354 (7), 221 (17), 214 (13), 204 (32), 201 (17), 191 (33), 175 (30), 136 (11), 135 (23), 123 (29), 43 (100).

12-Oxo-2β,3β,11α-tri-*O*-acetyl-5β-hydroxybufalin (8). Acetic anhydride (0.5 mL) was added to a solution of the diacetate **5** (53 mg, 0.1 mmol) in pyridine (1 mL) and the resulting solution heated at 60 °C for 2 h. After removal of the volatile material in vacuo, the residue was purified by column chromatography (silica gel; 3:3:4 acetone–chloroform–hexane) to afford the triacetate **8** (48 mg, 84%): mp 109–111 °C; ^1H NMR δ 0.96 (3, s), 1.18 (3, s), 1.3–2.4 (16, m), 1.97 (3, s), 2.07 (3, s), 2.13 (3, s), 4.06 (1, d of d, *J* = 9, 6 Hz), 5.20 (1, d of t, *J* = 12, 3, 3 Hz), 5.50 (1, d, *J* = 12 Hz), 5.57 (1, br), 6.30 (1, d, *J* = 10 Hz), 7.43 (1, d, *J* = 2 Hz), 7.77 (1, d of d, *J* = 10, 2 Hz); EIMS (70 eV) *m/e* (rel intensity) 574 (M^+, 1), 556 ($M^+ - H_2O$, 1), 514 (1), 496 (1), 454 (1), 412 (2), 394 (4), 376 (1), 220 (8), 205 (28), 204 (4), 191 (20), 175 (6), 136 (2), 135 (7), 123 (6), 43 (100).

Treatment of both monoacetates **6** and **7** with Ac_2O–pyridine in a similar manner afforded the same triacetate (TLC, LC, and ^1H NMR).

12-Oxo-2β,5β,11α-trihydroxybufalin (9). Diacetate **5** (100 mg, 0.2 mmol) was dissolved in methanol saturated with ammonia and the resulting solution stirred at room temperature for 4 h. Evaporation of the volatile material in vacuo afforded the deacetylated product **9** (84 mg, 95%). Purification by column chromatography (silca gel; 9:1 chloroform–methanol) gave **9** as a white solid (mp 168–170 °C) accompanied by a second component which appeared to be the 11-oxo-12-hydroxy isomer. **9**: ^1H NMR ($(CD_3)_2SO$) δ 0.80 (3, s), 1.03 (3, s), 1.1–2.4 (14, m), 3.7–4.2 (4, m, one H exchanges with D_2O), 4.38 (1, d of d, *J* = 12, 4 Hz, addition of D_2O simplifies to d, *J* = 12 Hz), 4.53 (1, d, *J* = 3 Hz, exchanges with D_2O), 4.98 (1, d, *J* = 3 Hz, exchanges with D_2O), 5.07 (2, s, exchanges with D_2O), 6.34 (1, d, *J* = 10 Hz), 7.61 (1, d, *J* = 2 Hz), 7.88 (1, d of d, *J* = 10, 2 Hz); EIMS (70 eV) *m/e* (rel intensity) 448 (M^+, 11), 430 ($M^+ - H_2O$, 16), 412 ($M^+ - 2H_2O$, 25), 404 (10), 394 (18), 387 (19), 369 (25), 214 (44), 204 (48) 201 (36), 191 (41), 175 (44), 149 (100), 136 (20), 135 (37), 123 (55), 79 (91) 43 (63).

12-Oxo-2β-*O*-acetyl-3β,11α-di-*O*-isobutyryl-5β-hydroxybufalin (10). Isobutyryl chloride (100 μL) was added dropwise to a solution of **6** (100 mg, 0.2 mmol) in pyridine (1 mL) and portions of the resulting solution were removed periodically for LC analysis. During the course of this reaction, the concentration of **6** decreased and a product with R_f identical with that of **11** was observed. As the reaction neared completion, only a second product of slightly longer R_f was detected. Evaporation of the volatile material in vacuo and purification of the residue by column chromatography gave **10** as a glass (95 mg, 74%). The ^1H NMR spectrum of **10** was very similar to that of the triacetate **8**, except that two acetate methyl group resonances were replaced by butyrate signals (δ 1.19 (6, d, *J* = 7 Hz), 1.20 (6, d, *J* = 7 Hz), 2.57 (2, sept, *J* = 7 Hz)); EIMS (70 eV) *m/e* (rel intensity) 630 (M^+, 0.1), 612 ($M^+ - H_2O$, 0.1), 394 (5), 204 (5), 201 (2), 191 (12), 175 (5), 135 (4), 123 (3), 71 (59), 43 (100).

Treatment of compound **11** with isobutyryl chloride under identical conditions also gave compound **10**, while the analogous reaction of **7** afforded a different compound.

12-Oxo-2β-*O*-acetyl-3β-*O*-isobutyryl-5β,11α-dihydroxybufalin (11). Compound **11** was obtained as a glass which resisted crystallization, although it appeared homogeneous on the basis of LC analysis: $[\alpha]_D^{EtOH}$ +27.0° (*c* 0.44); ^1H NMR δ 0.93 (3, s), 1.20 (3, d, *J* = 7 Hz), 1.21 (3, d, *J* = 7 Hz), 1.23 (3, s), 1.4–2.5 (16, m), 1.95 (3, s), 2.60 (1, sept, *J* = 7 Hz), 3.90 (1, br, exchanges with D_2O), 4.10 (1, d of d, *J* = 9, 6 Hz, 4.40 (1, br d, addition of D_2O simplifies to d, *J* = 12 Hz), 5.12 (1, d of t, *J* = 12, 3, 3 Hz), 5.56 (1, br), 6.30 (1, d, *J* = 10 Hz), 7.43 (1, d, *J* = 2 Hz), 7.78 (1, d of d, *J* = 10, 2 Hz); the ^{13}C NMR spectrum of **11** corresponded closely to that of **5**, except that the acetate ester absorptions at δ 169.2 and 20.7 were replaced by isobutyrate ester absorptions[22] at 175.2 (s, C=O), 34.0 (d, $(CH_3)_2CH$), and 18.8 and 18.4 (q, q, $(CH_3)_2CH$); EIMS (70 eV) *m/e* (rel intensity) 560 (M^+, 3), 542 ($M^+ - H_2O$, 6), 524 ($M^+ - 2H_2O$, 3), 500 (2), 482 (1), 464 (1), 412 (9), 394 (18), 376 (12), 366 (7), 354 (5), 348 (6), 221 (14), 204 (25), 201 (9), 191 (18), 175 (16), 136 (6), 135 (13), 123 (15), 71 (43), 43 (100).

Treatment of **11** (8 mg, 0.014 mmol) with isobutyryl chloride (75 μL) in pyridine for 4 h at room temperature afforded **10** (TLC, LC, and ^1H NMR).

12-Oxo-2β-*O*-acetyl-3β-*O*-propionyl-5β,11α-dihydroxybufalin

(**12**). Compound **12** was obtained as a glass which resisted crystallization, although it appeared homogeneous on the basis of LC analysis: $[\alpha]_D^{EtOH}$ +36.6° (*c* 0.45); ^{1}H NMR δ 0.92 (3, s), 1.19 (3, t, *J* = 7 Hz), 1.22 (3, s), 1.3–2.6 (18, m), 1.95 (3, s), 3.90 (1, br, exchanges with D_2O), 4.10 (1, d of d, *J* = 9, 6 Hz), 4.40 (1, br d, addition of D_2O simplifies to d, *J* = 12 Hz), 5.12 (1, d of t, *J* = 12, 3, 3 Hz), 5.56 (1, br d, *J* = 2 Hz), 6.30 (1, d, *J* = 10 Hz), 7.43 (1, d, *J* = 2 Hz), 7.80 (1, d of d, *J* = 10, 2 Hz); the ^{13}C NMR spectrum of **12** corresponds closely to that of **5**, except that the acetate ester absorptions at δ 169.2 and 20.7 are replaced by propionate ester absorptions[22] at 172.9 (s, C=O), 29.8 (t, CH_2), and 8.7 (q, CH_3); EIMS (70 eV) *m/e* (rel intensity) 546 (M^+, 1), 528 ($M^+ - H_2O$, 2), 394 (3), 376 (1), 221 (10), 204 (16), 191 (14), 175 (10), 136 (5), 135 (10), 123 (14), 57 (71), 43 (100).

12-Oxo-2β-*O*-acetyl-3β,11α-di-*O*-propionyl-5β-hydroxybufalin (16). Propionyl chloride (100 μL) was added dropwise to a solution of **6** (100 mg, 0.2 mmol) in pyridine (1 mL) and the resulting solution stirred at room temperature. Portions of the reaction mixture were removed for periodic analysis by LC. The initial reaction product corresponded in R_f to compound **12** and as the reaction progressed a second product of slightly longer retention time was observed. Evaporation of the volatile material in vacuo and purification of the residue by column chromatography gave **16** as a viscous oil (92 mg, 76%): the ^{1}H NMR spectrum of **16** was very similar to that of the triacetate **8**, with the resonances of two propionate esters (δ 1.16 (3, t, *J* = 7 Hz), 1.17 (3, t, *J* = 7 Hz), 2.35 (4, q, *J* = 7 Hz)) replacing the acetate resonances of **8**; EIMS (70 eV) *m/e* (rel intensity) 602 (M^+, 0.2), 584 ($M^+ - H_2O$, 0.2), 394 (5), 205 (17), 204 (7), 191 (15), 175 (6), 135 (6), 123 (4), 57 (100), 43 (35).

Treatment of compound **12** with propionyl chloride under identical conditions also gave **16**, while treatment of **7** with propionyl chloride gave a different compound.

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Identity of the Chain-Carrying Species in Halogenations with Bromo- and Chloroarylalkoxyiodinanes: Selectivities of Iodinanyl Radicals

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Abstract: The free-radical halogenation of substituted toluenes by haloiodinanes **2a–b** and **3a–b** in benzene solvent is highly selective for benzylic hydrogens. The process involves cyclic iodinanyl radicals, except in the case of **3b**, which appears to react via a bromine atom chain. The essentially identical values of ρ^+ for **2a** (−1.46) and **2b** (−1.48) are consistent with there being a common chain-carrying species for both bromination and chlorination. Identical ρ^+ values were not observed for **3a** (−1.31) and **3b** (−1.55 vs. Br, −1.58). Such iodinanyl radicals, unlike those derived from phenyliodine dichloride, are constrained to a C–I–O angle far smaller than 180°, allowing an opportunity to study the effects of bending on radical selectivities. The intermediacy of iodinanyl radicals in free-radicals chlorinations is further supported by evidence from photoinitiated reactions of **2a** and **3a** with 2,3-dimethylbutane. Comparisons of selectivities with those determined in other studies show that chlorine atoms are not involved. The allylic chlorinations of *cis*- and *trans*-2-butenes by **2a** and **3a** were studied and found to be selective, high-yield reactions which give little or no addition to the carbon–carbon double bond.

Introduction

Organic compounds of tricoordinate iodine(III) may be generally designated as hypervalent[1] iodine species and more specifically as iodinanes.[2] The formation of iodine-centered radical intermediates from such iodinanes, since it involves breaking weak bonds to iodine, is expected to be an energetically favorable process.

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