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ENOLIC IRIDOLACTONE AND OTHER IRIDOIDS FROM *ALBERTA MAGNA*

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Key Word Index—*Alberta magna*; Rubiaceae; iridoids; iridolactone; cyclopentene dialdehyde; mosquito repellent.

Abstract—From fresh leaves of *Alberta magna* two new iridoids and a known cyclopentene dialdehyde have been identified. One of the new compounds has the less-common irido-lactone structure and has an enolic hydrogen on C-4. Two of the compounds show short term mosquito-repellent effects. © 1998 Published by Elsevier Science Ltd. All rights reserved

INTRODUCTION

Alberta magna E. Mey. is a small tree with a very restricted distribution. Only five genera occur worldwide and these are limited to South Africa and Madagascar [1]. *A. magna* is found in the province of KwaZulu-Natal with small stands in Pondoland (Eastern Cape Province). The tree in question, *A. magna* E. Mey., is the type specimen on which the genus was founded [2]. It is not easily cultivated in gardens and prefers to grow wild on forest margins and on rocky outcrops. There are vague references to its use as a 'muthi' (medicine) among the Zulu people [3] but no specific application is recorded. There is, however, no doubt that substantial quantities of the bark are utilized by the traditional healers in northern KwaZulu-Natal.§

Our interest in the tree was first aroused by the observation that trees grown in cultivation in the Pietermaritzburg area were remarkably free of insect infestation. Working on the assumption that it possibly contained an insect anti-feedant or insect-repellent principle, its leaf extractives were examined. This working hypothesis was strengthened when it was observed that TLC plates of the crude extract afforded a typical pink colouration when treated with the standard 4-anisaldehyde-sulphuric acid reagent. Similar colourations had been observed by us while examining the organic extracts of *Warburgia salutaris* [4]. The bark of this tree, which occurs locally, contains warburganal (**1**), and other dialdehydes which exhibit insect anti-feedant properties [5]. This was the first clue that we might be dealing with compounds in *A. magna* bearing one, or more, free aldehyde groups.

RESULTS AND DISCUSSION

In the present investigation, two new iridoids and a known dialdehyde have been isolated and fully characterized. The last, 5-acetaldehyde-1-formyl-2-methylcyclopent-1-ene (**2**), formally the decarboxylated form of deoxyloganin aglucone, has been reported previously in the Japanese patent literature [6], as a constituent of *Vitex rotundifolia* (Verbenaceae). The Japanese patent refers to the use of the aldehyde as a repellent for noxious and blood-sucking insects. To our knowledge details of the dialdehyde appears only in the Japanese language and for this reason some detail regarding the compound isolated from our investigation will be given. It should be noted that the specific rotation of our compound, $[\alpha]_D+81.2°$, is more than double the rotation found by the Japanese researchers.

The two new iridoids reported here are 1,4a,5,6,7a-hexahydro-1-hydroxy-7-methyl-cyclopenta[*c*]pyran-4-carboxaldehyde (**3**), (a minor constituent), and 4, 4a, 5,7a-tetrahydro-1-hydroxy-4-(hydroxymethylene)-7-methylcyclopenta[*c*]pyran-3-(1H)-one (**4a**).‖

Careful scrutiny of the older literature revealed that as far back as 20 years ago Bianco [7] had isolated **2** from the hydrolysis of the naturally-occurring iridoid glucoside 6,10-*bis*-deoxyaucubin (**5**). In this instance

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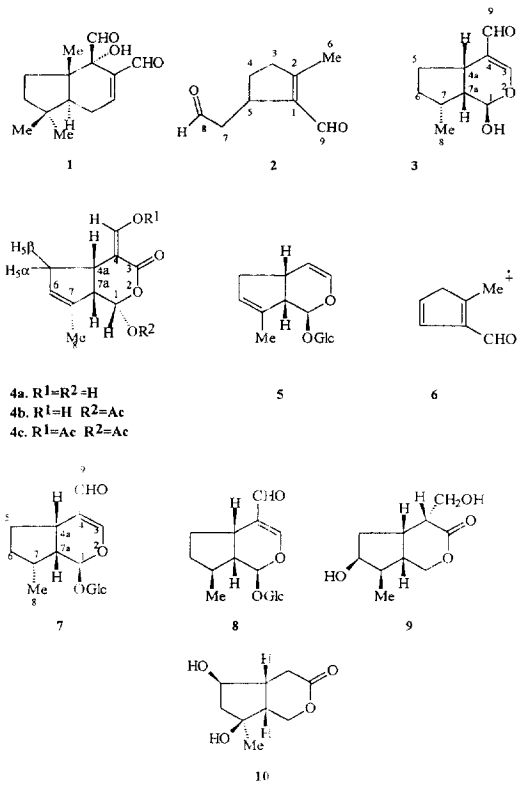

only small quantities of **2** were isolated for which the IR spectrum is given. Other than the chemical shifts for the two aldehydic protons and the position of the allylic methyl signal, no other spectroscopic information is quoted by Bianco [7].

The structure of **2** follows directly from the ^1H and ^{13}C NMR data coupled with the normal COSY and HETCOR pulse sequences. Spin-spin-decoupling experiments were found to be particularly useful to establish the relationships between relevant protons. Superficially the signals resulting from the protons on C-3, C-4, C-7 and the vinylic methyl group appear as a complex multiplet between δ 1.5 and 2.90.

The mass spectrum of **2**, apart from showing a definite $[M]^+$ peak at *m/z* 152, also has a diagnostic peak at *m/z* 108. This prominent fragment almost certainly arises by a loss of 44 amu from the $[M]^+$ peak via a McLafferty rearrangement to afford ion **6** at *m/z* 108.

Iridoid **3** is new in the sense that previously it has only been found as the glucoside [8, 9]. Its structure could be established by analysis of the HETCOR and COSY NMR spectra. It was not a simple matter to assign the individual protons present in the cyclopentane ring since extensive coupling between protons on adjacent carbons leads to a complex set of multiplets. Mass spectrometry confirmed the proposed structure. The $[M]^+$ is at *m/z* 182 and high resolution analysis established the composition of the ion to be $C_{10}H_{14}O_3$.

The problem of assigning the stereochemistry of the methyl group at C-7 was solved by referring to the published data on the diastereomeric glucosides of **3** which occur in Nature. These are boschnaloside 7 [8,9] and 5-deoxy-stansioside **8** [10] which are present in *Leucocarpus perfoliatus* (Scrophulariaceae) and *Tecoma stans* (Bignoniaceae), respectively. Comparison of the proton and ^{13}C spectra recorded by Jensen and his coworkers [11] for **7** and **8** and many related iridoids, shows that the chemical shift of the α-methyl group in this series occurs typically at about 16 ppm and C-7a at about 43 ppm. The corresponding chemical shift values for the β-methyl compounds are at 20 and 49 ppm, respectively. Extrapolation of these findings to our own indicates that aglucone **3** is indeed the α-Me isomer since the carbons in question resonate at 16.4 and 44.4 ppm, respectively.

The major constituent of *A. magna* leaves was shown to be 4,4a,5,7a-tetrahydro-1-hydroxy-4-hydropxyethylene-7-methylcyclopenta[*c*]pyran-3(1H)-one (**4a**). For the sake of brevity, and in keeping with iridoid usage, the compound is designated amagnalactone.

Establishing the structure proved to be a daunting task since it posesses an aldehyde group in the enolic form; an unusual arrangement unless the vinyl hydroxyl group has good reason to exist in this tautomeric form. In this instance the reason is the adjacent ester carbonyl group which permits H-bonding.

Evidence in favour of the above follows: The IR spectrum of **4a** indicated an H-bonded lactone moiety at 1684 cm^{-1}. This peak shifted to 1713 cm^{-1} in the monoacetyl derivative **4b** (acetylation at C-1) and in the diacetate **4c** the peak appeared at 1740 cm^{-1}, a value typical of the carbonyl in a 6-ring lactone. Further evidence came from the proton and ^{13}C spectra (see below).

The mass spectrum of **4a** showed no $[M]^+$ peak. However, silylation afforded the $[M]^+$ at *m/z* 340. A puzzling feature of the mass spectrum was the prominent peak at *m/z* 268. From the subsequent NMR spectral evidence, it was concluded that amagnalactone (**4a**) was a dihydroxy compound and that silylation afforded mainly the disilyl derivative (*m/z* 340), but that some monosilyl product was also present (*m/z* 268). These results were confirmed through preparation of the diacetate derivative which has a prominent mass peak at *m/z* 220, corresponding to an $[M\text{-}60]^+$ fragment. Accurate mass analysis showed the composition of this peak to be $C_{12}H_{12}O_4$ so that the diacetate had $[M]^+$ = *m/z* 280. Accordingly, the parent compound has the molecular composition $C_{10}H_{12}O_4$ $[M]^+$ = *m/z* 196.

Acetylation studies were invaluable in assigning the final structure. Since the unacetylated compound, **4a**, was always contaminated with traces of other isomers (epimers), the monoacetate **4b** (mild conditions) and the diacetate **4c**, were also prepared. These could be obtained in pure form. Comparison of the NMR spectral data (COSY, HETCOR) for these three derivatives gave a consistent overall picture. As an example

Table 1. Comparison of proton shifts for selected protons in **4a**, **4b** and **4c** ($CDCl_3$ solvent)

H	**4a** Unacetylated	**4b** Monoacetate	**4c** Diacetate
-1	4.90(*d*, J = 8.0 Hz)	5.95(*d*, J = 6.5 Hz)	6.60(*d*, J = 2.9 Hz)
-9	7.59(*d*, J = 0.9 Hz)	7.55(*d*, J = 1.0 Hz)	8.24(*d*, J = 2.0 Hz)

Table 2. Effect of irradiating selected protons in **4c**

Proton irradiated (δ_H)	Proton(s) affected (chem. shift)
Me (1.80)	H-5α(2.86), H-6(5.54, homoallylic coupling), H-7a(3.00)
H-4a (3.64)	H-5α(2.86), H-7a(3.00), H-9(8.24)
H-6 (5.54)	Me(1.80), 5α(2.86)
H-1 (6.60)	H-7a(3.00)
H-9 (8.24)	H-4a(3.64)

of this, the proton chemical shifts of H-1 and H-9 are highlighted in Table 1.

Since the COSY data did not allow for satisfactory assignment of all the overlapping proton signals, systematic spin decoupling was undertaken. This technique was very successful and from irradiation of key protons in **4c** the following observations, consistent with the proposed structure, could be made (Table 2).

NOE studies were carried out on **4a**, **4b** and **4c** and the strongest correlations are indicated in Fig. 1. Much weaker correlations were detected between H-1 and H-9 and between H-1 and H-5β in both **4a** and **4b**. It is important to note the strong NOE correlation between H-4a and H-7a (as is common in all iridoids) as well as that between H-7a and H-1 (see below).

The results from an analysis of the proton NMR spectra of **4a**, **4b** and **4c**, coupled with the observed NOE between H-7a and H-1, allow some deductions to be made regarding the stereochemistry of the 6-membered lactone. In **4a** the $J_{1,7a}$ value is 8.0 Hz, in **4b** (monoacetate) it decreases to 6.5 Hz, and in **4c** (diacetate) it is only 2.9 Hz. We believe these observations are best accommodated by a lactone ring in which the protons on C-4a and C-7a retain the 'normal' β-configuration and are *cis* to one another. However, the hydroxyl group at C-1, in this instance, is *trans* (and eq.) to the two protons in question. Obviously this arrangement places the proton on C-1, *cis* relative to the protons on C-4a and C-7a. If one further assumes that the lactone ring in **4a** adopts a conformation in which O-2, C-3, C-4 and C-4a are coplanar, and that for **4b** and **4c** the conformation tends towards a twist boat form, then it can be seen that the decrease in J value from 8.0–6.5–2.9 Hz is consistent with dihedral angles of the order of 18 to 30 to 55°. The change in dihedral angle is possibly the result of the acetylation process.

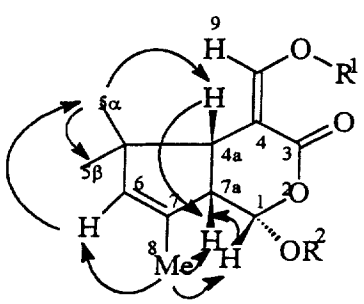

Fig. 1.

It is difficult to compare the conclusions above with the results published for other iridolactones, specifically alyxialactone **9**, isolated from *Alyxia reinwardti* by Cordell and coworkers [12]. This iridolactone has a hydroxymethyl group at C-4 but this does not have the same restricting influence on the conformation of the ring as the hydroxyethylene functionality in **4a**. The latter readily assumes a boat conformation but does not accommodate a chair conformation, as is postulated for alyxialactone **9**. Accordingly, the size of dihedral angles are quite markedly different.

Another iridolactone, for which an X-ray structure is available, is jioglutolide (**10**), from *Rehmannia glutinosa*. In this instance, Morota *et al.* [13] assume the 'normal' configuration of iridoids (see above) and the X-ray analysis depicts a $^1B_4$ boat conformation for the lactone ring.

The three iridoids reported here fit in extremely well with the accepted biosynthetic pathway of the iridoids [14, 15]. Isolation of **3** in the form of its aglycone represents an important step in the overall pathway. The iridotrial can be regarded as the common precursor for the iridoids described here (Fig. 2). This proposed interrelationship of the three iridoids is also in keeping with our observations that in late winter the composition of the bark extract was markedly different from that observed in mid summer. In the latter season almost none of **3** could be detected and the concentration of **4** was also considerably reduced. It should be noted that the compounds described here could indeed be artefacts arising from enzymatic cleavage of the glucosides present in the plant. This point requires further study.

Biological tests

Two assays for mosquito repellency were carried out on **2**, and **4a** was tested by one of these procedures. In both instances a count is done of the number of unfed female mosquitoes which settle on a fixed area of human skin after a specific period of time. DEET (*N*,*N*-diethyl-3-methylbenzamide, or diethyltolu-

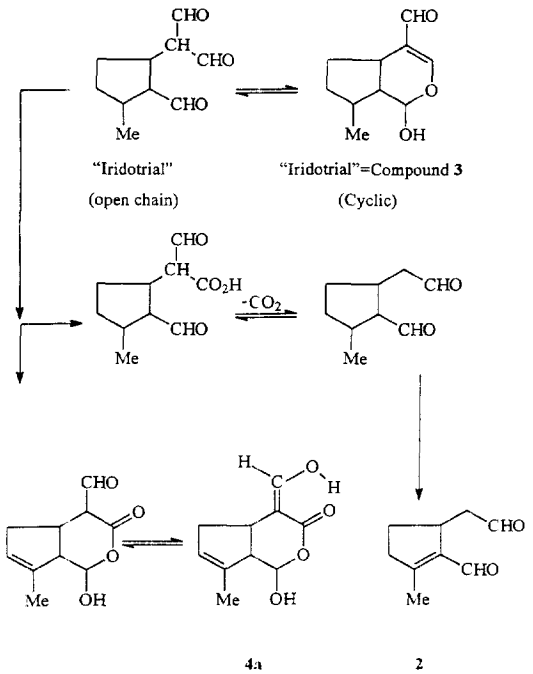

Fig. 2.

Table 3. Mosquito repellency tests on compound **2**

Day	Compound **2**	DEET	Control
1	0, 0, 0	0, 0, 0	3+, 3+, 3+
2	2, 1, 3	0, 1, 1	3+, 3+, 3+
3	3+, 3+, 3+	0, 0, 0	3+, 3+, 3+
4	3+, 3+, 3+	0, 0, 1	3+, 3+, 3+

amide) was used as reference compound. The results are shown in Table 3.

The figures represent the number of mosquitoes feeding on the test patch for three consecutive 1 min intervals.

In order to counteract the high volatility of the test substances, the formulations were made up as an unperfumed lotion. In Table 4 results are shown for the modified procedure.

It can be concluded that the two test substances from *A. magna* do have a repellent effect on the mosquitoes tested, but that this effect is short-lived. All these test results were performed on *Anopheles arabiensis* (a malaria vector) and then repeated on *Aedes aegypti* (a common nuisance mosquito) and similar results were obtained. Variation in formulation to extend the 'residence time' of the test substances on the exposed area may effect some improvement, but this remains to be done.

Table 4 Modified mosquito repellency tests on compounds **2** and **4a**

Time after treatment (hr)	Compound **2**	Compound **4a**	DEET
1	0	3	0
2	18	11	0
3	26	23	5

EXPERIMENTAL

General. NMR: ^{1}H (200 MHz) and ^{13}C (50 MHz); EI-MS: 70 eV; CC: silica gel 60 (Macherey Nagel); Chromatotron: silica gel 60F_{254}.

Plant material. Alberta magna E. Mey. Leaves were collected in July 1996 in the National Botanical Garden in Pietermaritzburg from a mature tree, identified and authenticated by the Curator of the Gardens, Mr Brian Tarr. A voucher specimen was deposited in the University of Natal Herbarium, Pietermaritzburg (Drewes No 1).

(+)-5-*Acetaldehyde*-1-*formyl*-2-*methylcyclopent*-1-*ene* (**3**). Freshly collected leaves (922 g) were extracted with CH_2Cl_2 at room temp. (12.1 g) and subsequently with EtOAc (11.8 g). The entire CH_2Cl_2 extract was fractionated by CC (silica gel, CH_2Cl_2) and subsequently on the chromatotron (CH_2Cl_2) to afford an oil (160 mg), $[\alpha]_D^{22}$ +81.2 (lit +39.3° [6]) ($CHCl_3$, *c*0.75). GCMS *m/z* (rel. int): 152 $[M]^+$(1), 134(23), 124(16), 108(82), 95(42), 70(100): ^{1}H NMR ($CDCl_3$, 200 MHz): δ1.50 (1H, *dddd*, *J* = 5.3, 4.6, 3.7 Hz, H-4α), 2.15 (1H, *m*, H-4β), 2.16 (3H, *dd*, *J* = 1.4, 1.3 Hz, Me), 2.34 (1H, *ddd*, *J* = 2.2, 9.3, 16.8 Hz, H-7α), 2.56 (2H, *m*, H-3), 2.94 (1H, *ddd*, *J* = 1.6, 4.2, 16.8 Hz, 7β), 3.47 (1H, *m*, H-5), 9.76 (1H, *dd*, *J* = 1.6, 2.2 Hz, H-8), 9.93 (1H, *s*, H-9); ^{13}C NMR ($CDCl_3$, 50 MHz): δ14.5(C-6), 28.2(C-4), 38.2(C-5), 39.0(C-3), 47.8(C-7), 139.1(C-1), 164.0(C-2), 188.0(C-9), 202.1(C-8)

1,4*a*,5,6,7*a*-*Hexahydro*-1-*hydroxy*-7-*methyl*-*cyclopenta*[*c*]*pyran*-4-*carboxaldehyde* (**3**). Leaves (Winterskloof area) of *A. magna* (authenticated as before) (660 g) were first extracted with CH_2Cl_2 (10.1g) and then with EtOAc (4.2 g). The CH_2Cl_2 extract was purified on short (5 cm) using CH_2Cl_2 initially, followed by hexane–EtOAc(1:1) to give a brown solid (170 mg). Final purification on the chromatotron, hexane–Et_2O(1:1), afforded an oil (24 mg), $[\alpha]_D^{25}$ +54.6° ($CHCl_3$, *c* 0.161). GCMS *m/z* (rel. int): $[M]^+$(20), 153(23), 136(30), 112(21), 107(39), 94(59), 71(98), 55(100): HR-MS: *m/z* 182.0939 ($C_{10}H_{14}O_3$ requires 182.0942); GCMS of TMS derivative, *m/z* (rel. int): 254$[M]^+$ (2.2): ^{1}H NMR ($CDCl_3$, 200 Mhz): δ1.13 (3H, *d*, *J* = 7.2 Hz, Me), 1.30 (1H, *m*, H-6β), 1.35 (1H, *m*, H-5β), 2.06 (1H, *ddd*, *J* = 7.3 Hz, H-7a), 2.30 (1H, *m*, H-6α), 2.80 (1H, *m*, H-5α), 2.90 (1H, *m*, H-4a), 5.25 (1H, *d*, *J* = 7.1 Hz, H-1), 7.20 (1H, *s*, H-3), 9.73 (1H, *s*, H-9); ^{13}C NMR ($CDCl_3$, 50 MHz): δ16.4(Me), 30.4(C-6), 31.7(C-5), 32.5(C-4a), 36.4(C-7), 44.4(C-7a), 95.9(C-1), 127.4(C-4), 161.7(C-3).

Isolation of compound **4a**. This compound occurred in the highest concn in leaves from an *A. magna* tree growing in a private garden in the Winterskloof area

of Pietermaritzburg. It was authenticated as before. Plant material (200 g) was collected in May 1996 and successively extracted with CH_2Cl_2 (1.9 g) and EtOAc (1.5 g). Purification of the latter extract by CC {CH_2Cl_2 followed by hexane–EtOAc, (3:2)] and the chromatotron using the same solvent mixt, afforded (**4a**). named amagnalactone, as an oil (40 mg).

(−)-4,4a,5,7a-*Tetrahydro*-1-*hydroxy*-4-(*hydroxymethylene*)-7-*methylcyclopenta*[c]*pyran*-3-(1*H*)-*one* (**4a**) * The oil had $[\alpha]_D^{20}+138°$ ($CHCl_3$, *c* 0.19). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3050, 3000, 1684(H-bonded C=0), 1616, 1116; GCMS of the TMS derivative *m/z* (rel. int): 340[M]$^+$(5), 250(63), 235(38), 180(92), 160(100), 132(7), 73(55); ^{1}H NMR ($CDCl_3$, 200 MHz): δ1.85 (3H, *d*, *J* = 1.4 Hz, Me), 2.10 (1H, *m*, H-5β), 2.40 (1H, *dd*, *J* = 8.0, H-7a), 2.80 (1H, *m*, H-5α), 3.16 (1H, *m*, H-4a), 4.90 (1H, *d*, *J* = 8.0 Hz, H-1), 5.54 (1H, *d*, *J* = 1.3 Hz, H-6), 7.59 (1H, *d*, *J* = 0.9 Hz, H-9); ^{13}C NMR ($CDCl_3$, 50 MHz): δ16.5(Me), 35.4(C-4a), 38.9(C-5), 49.9(C-7a), 96.8(C-1), 110.5(C-4), 127.5(C-6), 139.1(C-7), 154.2(C-9), 173.2(C-3). The compound was unstable and conversion to the acetate derivative(s) was undertaken in an attempt to stabilize it.

Acetylation of amagnalactone (**4a**). Amagnalactone (4a) (90 mg) was acetylated at room temp. (12 h^{-1}) with pyridine and Ac_2O. Isolation from H_2O gave a product which showed two major spots on TLC (R_f 0.4, 0.5, CH_2Cl_2). Subsequent purification on the chromatotron (CH_2Cl_2) yielded the mono- and di-acetyl derivatives.

(+)-4,4a,5,7a-*Tetrahydro*-1-*acetoxy*-4-(*hydroxymethylene*)-7-*methylcyclopenta*[c]*pyran*3-(1H)-*one* (**4b**). The monoacetyl derivative from above (14 mg) was an oil $[\alpha]_D^{22}+100°$ ($CHCl_3$, *c* 0.007). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3060, 2860, 1758, 1713(H-bonded C=0), 1635, 1104, 1244 and 1196; ^{1}H NMR ($CDCl_3$, 200 MHz): δ1.77 (3H, *t*, *J* = 0.7 Hz, Me), 2.16 (3H, *s*, OAc), 2.05–2.22 (1H, *m*, H-5α), 2.70 (1H, *m*, H-7a), 2.82 (1H, *m*, H-5β), 3.22 (1H, *m*, H-4a), 5.54 (1H, *d*, *J* = 1.5 Hz, H-6), 5.95 (1H, *d*, *J* = 6.5 Hz, H-1), 7.55 (1H, *d*, *J* = 1.0 Hz, H-9); ^{13}C NMR ($CDCl_3$, 50 MHz): δ15.7(C-8), 21.0(OAc), 33.9(C-4a), 38.5(C-5), 47.8(C-7a), 91.9(C-1), 111.0(C-4), 128.3(C-6), 137.1(C-7), 153.6(C-9), 169.5(OAc), 172.6(C-3).

(+)-4,4a,5,7a-*Tetrahydro*-1-*acetoxy*-4-(*acetoxymethylene*)-7-*methylcyclopenta*[c]*pyran*-3-(1H)-*one* (**4c**). The diacetyl derivative (24 mg) was an oil $[\alpha]_D^{22}$ +46.3° ($CHCl_3$, *c* 0.1). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1784, 1780, 1740, 1652, 1608; GCMS *m/z* (rel. int): 220[M-60]$^+$(21), 192(10) 178(21), 150(85), 122(47), 108(14), 80(100), 53(9); HR-MS: *m/z* 220.0731 ($C_{12}H_{12}O_4$ [M-60]$^+$ requires 220.0735); ^{1}H NMR ($CDCl_3$, 200 MHz): δ1.80 (3H, *s*, Me), 2.11 (3H, *s*, OAc), 2.28 (3H, *s*, OAc), 2.16–2.26 (1H, *m*, H-5α), 2.86 (1H, *m*, H-5β), 3.00 (1H, *m*, H-7a), 3.64 (*btt*, H-4a), 5.52 (1H, *sm*, H-6), 6.60 (1H, *d*, *J* = 2.9 Hz, H-1), 8.24 (1H, *d*, *J* = 1.9 Hz, H-9); ^{13}C NMR ($CDCl_3$, 50 MHz): δ14.8(C-8Me), 20.6(OAc), 20.9(OAc), 32.9(C-4a), 39.7(C-5), 48.7(C-7a), 91.6(C-1), 116.0(C-4), 128.3(C-6), 136.3(C-7), 145.2(C-9), 166.1(2x OC*O*CH$_3$), 168.7(C-3).

Biological tests. Cotton gauze patches (50 cm^2) were impregnated with a soln of the test compounds in EtOH (50 mg 0.75 ml^{-1}) and allowed to dry. The cotton patches were then taped over a 'window'cut in a surgical rubber glove. A negative control in which the cotton was treated with EtOH only was prepd. Bioassays were carried out daily by donning the glove and inserting the gloved hand into a cage containing about 1000 unfed mosquitoes (*Anopheles arabiensis*) for three consecutive one-min intervals. The number of mosquitoes feeding was recorded. If more than 3 mosquitoes fed before the end of a minute they were manually disturbed and the next period started. Results are collected in Table 3.

For the second assay a circular area (65 cm^2) was marked on a human forearm. The test substances, **3** and DEET, were made up as 10 and 1% formulations, respectively, in unperfumed lotion, and applied evenly over the marked area. In practice this meant that 25 mg of **3** and 2.5 mg of DEET were used per application. In view of the small quantity of **4a** which was available, only 7 mg for each test, was applied to the marked area.

Paper cuts (500 ml) were prepd for each biting assay by cutting out the bases and replacing them with transparent film. Nylon gauze was used to close the mouth of the cups. The assay procedure involved inverting the cup (gauze cover down, and containing 30 unfed adult female mosquitoes of *Anopheles arabiensis*) onto the treated area of skin, while observing mosquito activity through the transparent base for 2 min. Results are in Table 4.

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