Tetrahedron Letters,Vol.23,No.10,pp 1039-1042,1982 0040-4039/82/101039-04$03.00/0
Printed in Great Britain

STEROIDAL ANTIFEEDANTS FROM THE DORID NUDIBRANCH ALDISA SANGUINEA COOPERI

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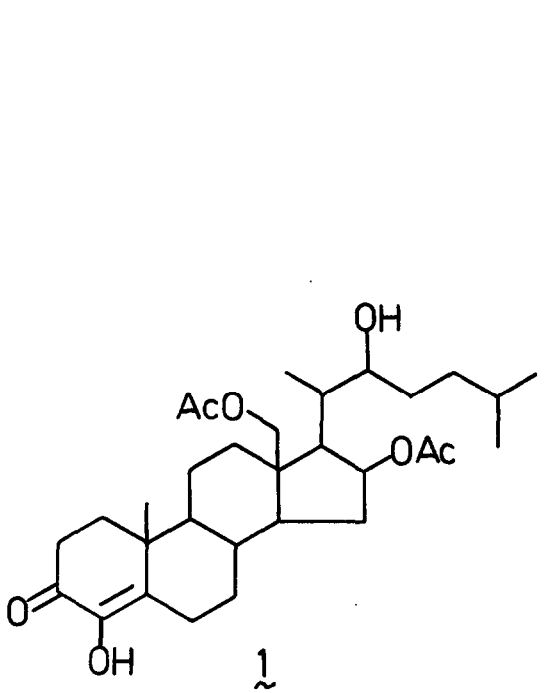
Summary: Two steroids, 3-oxo-chol-4-ene-24-oic acid (2) and its unsaturated analog 4, have been isolated from the dorid nudibranch Aldisa sanguinea cooperi, and the acid 2 has been shown to have antifeedant properties.

A number of recent studies have demonstrated that dorid nudibranchs utilize organic metabolites obtained from their diets as chemical defenses against predation.[1] The majority of the antifeedants discovered to date have been sesquiterpenoids. Numerous other interesting metabolites, to which no defensive role is ascribed, have also been isolated from nudibranch skin extracts.[2] The highly oxygenated steroid 1 from Hervia peregrina, Flabellina affinis and Coryphella lineata[3], and the 5α, 8α-epidioxysteroid mixtures from Adalaria sp.[4b] are examples. As part of an ongoing program to study the chemistry of nudibranch skin extracts [4,1c], we have examined Aldisa sanguinea cooperi. We report here the structures of two steroidal ketones from A. sanguinea cooperi extracts which contain bile acid side chains. The major steroid 2 displays significant antifeedant activity in a standard goldfish bioassay.

A. sanguinea was collected by hand using SCUBA (-1 to -6m) in Barkley Sound, British Columbia. The nudibranchs were usually found deeply embedded in the sponge Anthoarcuata graceae from which they obtain cryptic pigmentation and nutrition. Freshly collected specimens were extracted whole in methanol. Silica gel column and PTLC chromatography of the ethyl acetate soluble portion of the methanol extracts gave one fraction that contained a complex mixture of steroidal ketones having standard alkyl side chains and a second fraction containing pure acid 2 (≈2 mg/animal).

The IR spectrum of 2 (KBr) showed a broad OH stretch (3600 to 2400 cm^{-1}), two carbonyls (1720 and 1700 cm^{-1}) and a >C=C< stretch (1640 cm^{-1}) suggesting carboxylic acid, ketone and olefin functionalities. Treatment of the acid 2 with diazomethane converted it quantitatively to the methyl ester 3. A HRMS of ester 3 showed an intense parent ion at m/z 386.2821 (calcd 386.2821) appropriate for a molecular formula of $C_{25}H_{38}O_3$. A facile loss of C_2H_2O in the mass spectrum, in conjunction with a significant peak at m/z 271 (M^+ - side chain)

indicated a 3-oxo, Δ^4 nucleus. The ^{13}C NMR ($CDC\ell_3$, 100 MHz) of 3, which contained resonances at δ 199.4 (C3), 123.8 (C4), and 171.3 (C5), and the UV spectrum of 3 (λ_{max} 240 nm, ε 15,000 MeOH) supported this assignment.[6]



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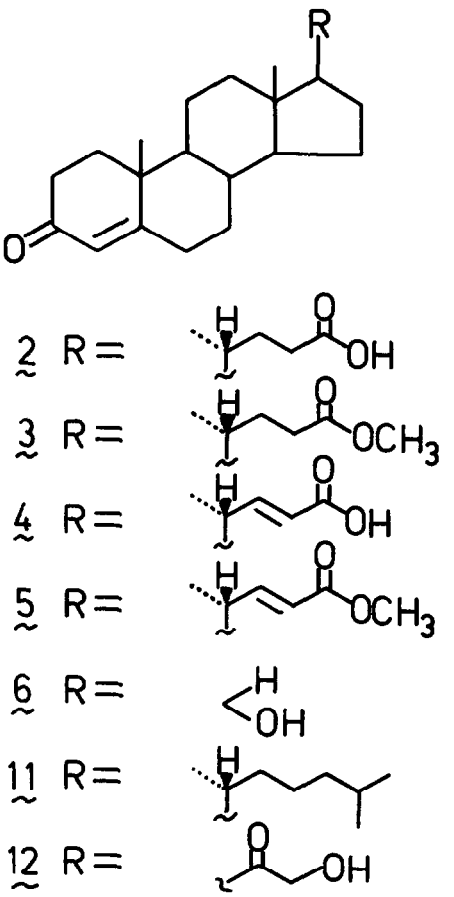


We next turned our attention to the side chain of 3 which must have a formula of $C_6H_{11}O_2$ and which must incorporate a methyl ester functionality. A methyl doublet (δ 0.93, J=6Hz) in the ^1H NMR revealed that C21 was not oxidized. The remaining methyl resonances in the ^1H NMR could be assigned to C19 (δ1.19), C18(0.72) and the methyl ester (3.68) indicating that there was no additional branching in the side chain. A saturated bile acid side chain is the only substructure consistent with the above evidence and hence 2 must be 3-oxo-chol-4-ene-24-oic acid[7].

One collection of A. sanguinea cooperi contained a mixture of 2 and the unsaturated acid 4 (≈3:1) which were separated via HPLC (2.4% isopropanol/hexane, Partisil PXS 5) as their methyl esters 3 and 5. The ester 5 showed[7]: HRMS M^+ 384.2655 (calcd 384.2664 for $C_{25}H_{36}O_3$), 342(M^+-C_2H_2O), and 271 (M^+ - side chain): UVλ_{max} 239, 214 nm (MeOH): ^1H NMR ($CDC\ell_3$, 400 MHz)

δ0.75, s, 3H, C18; 1.10, d, J=7 Hz, 3H, C21; 1.19, s, 3H, C19; 3.74, s, 3H, $-CO_2\underline{CH_3}$; 5.74, s, 1H, C4; 5.76, d, J=16 Hz, 1H, C23; 6.84, dd, J=16, 9Hz, 1H, C22.

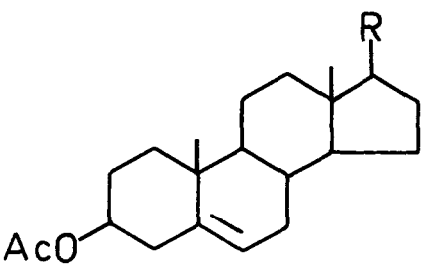
Vanderah and Djerassi have recently reported that the sea pen Ptilosarcus gurneyi contained methyl-(E)-3β-acetoxy-$\Delta^{5,22}$-choladienate (9) which had the unexpected 20S stereochemistry.[8] Unambiguous synthesis of a series of 20S steroids enabled them to demonstrate that the C21 methyl group is shifted ~0.1 ppm upfield in the ^{1}H NMR spectrum of the 20S compounds. Careful examination of the chemical shifts of the C21 methyl group in esters 3 and 5 proves that they both have the more common 20R configuration (Table 1).

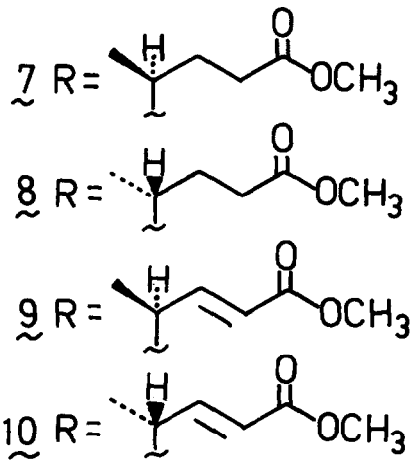
Table 1 ^{1}H NMR Data for Various 20R and 20S Steroids.

Compound	7	8	3	9	10	5
Chemical shift of C21	0.84[a]	0.94[a]	0.93[b]	1.00[a]	1.10[a]	1.10[b]

a) 100 MHz, $CDCl_3$, see ref. 8

b) 400 MHz, $CDCl_3$





We have examined the extracts of the sponge Anthoarcuata graceae[11] in an attempt to establish a dietary origin for the acids 2 and 4. The sponge, however, contains neither 2 or 4 but it does contain the same mixture of steroidal ketones with standard alkyl side chains that is found in A. sanguinea cooperi. Cholestenone (11) is the major component of this mixture.

A standard goldfish bioassay was used to test the antifeedant properties of 2 and 11.[1b] Our results showed that the acid 2 effectively inhibited feeding, while cholestenone (11) was totally inactive.[9] The nudibranch is apparently obtaining an inactive metabolite from its diet and chemically modifying it to produce an active antifeedant. It is interesting to note the structural similarities between 2 and 12; the latter compound is utilized as a fish repellent by the great diving beetle Dytiscus marginalis.[10]

Acknowledgements: We wish to acknowledge financial support from the Natural Sciences and Engineering Research Council of Canada in the form of a grant to RJA and a postgraduate scholarship to SWA. The authors wish to thank Mike Le Blanc and the staff of the Bamfield Marine Station for assistance in collecting A. sanguinea cooperi.

Footnotes and References

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2. For example see:
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6. Our data for 3 are virtually identical to the values reported for the corresponding functionalities in testosterone (6). Hanson, J.R.; Siverns, M. J.C.S. Perkin I, 1975, 1956.

7. The acids 2[a] and 4[b] are known synthetic compounds but they have not been previously reported from natural sources. Our physical data for both compounds are in agreement with the literature values.
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9. 2 was active at $<15\,\mu g/mg$ 11 was inactive at $>100\,\mu g/mg$.

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11. The sponge was identified by Dr. W. Austin who is in the process of reclassifying this species.

(Received in USA 17 November 1981)