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SYNTHESIS AND *IN VITRO* ANTIBACTERIAL ACTIVITY OF NEW SEMI-SYNTHETIC NOVIOSYLCOUMARIN ANTIBIOTICS: CHEMICAL MODIFICATION AT THE C-3' ESTER

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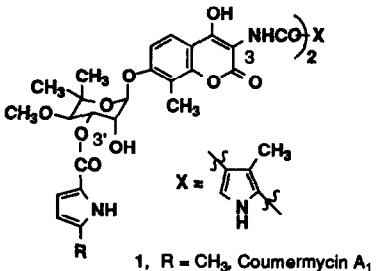
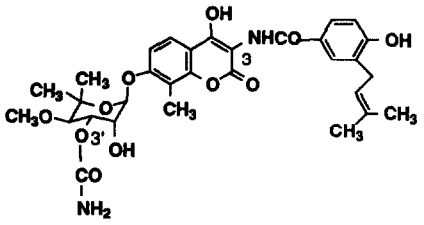
Abstract: A synthetic procedure to introduce a variety of ester groups at the C-3' position of the noviosylcoumarin antibiotics has been developed. Several new semi-synthetic noviosylcoumarins having unique ester groups at the C-3' position by this method were prepared and *in vitro* antibacterial (anti-staphylococcal) activity was evaluated.

Continued interest has been maintained in the noviosylcoumarin antibiotics,[1] such as coumermycin A_1 (**1**) and novobiocin (**2**), for two major reasons. This class of antibiotics, particularly coumermycin A_1 (**1**) possesses potent antibacterial activity against methicillin-resistant strains of staphylococcus species, which have become clinically important pathogens over the last decade.[2] Additionally, the mechanism of action, inhibiting the bacterial DNA gyrase,[3] associated with this class of antibiotics is unique.

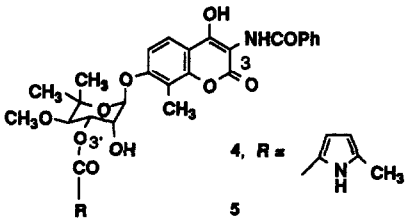
Although extensive studies on the chemical modification of coumermycin A_1 (**1**) have been reported, most of them reflect modifications at the C-3 position of the coumarin moiety.[4] Apparently very little effort has been directed towards the modification of the C-3' position of the noviose portion of coumermycin A_1 (**1**). It has been reported that coumermycin A_2 (**3**), which differs from coumermycin A_1 by having a pyrrole-2-carboxylate moiety instead of a 5-methylpyrrole-2-carboxylate group at the C-3' position, possessed much reduced antibacterial activity as compared to coumermycin A_1.[4a] Therefore, it appears that the substituent at this C-3' position plays an important role in determining the overall antibacterial profile of the particular coumermycin derivatives. In order to study the effect of the C-3' ester substituents on antibacterial activity, we have chosen to modify the monomeric derivative of coumermycin A_1, noviosylcoumarin **4**. This derivative **4** was used previously as a standard for the antibacterial evaluation of semi-synthetic coumermycin derivatives.[4] Herein, we report a synthetic process to noviosylcoumarin **5** having a variety of ester substituents at the C-3' position, and their *in vitro* antibacterial activity.



1, R = CH_3, Coumermycin A_1
3, R = H, Coumermycin A_2



2, Novobiocin

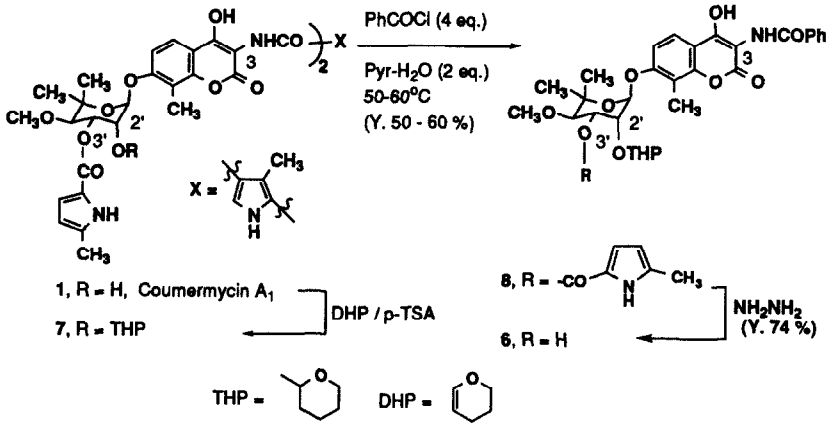


4, R =

5

The requisite intermediate for the modification at the C-3' position of the noviosylcoumarin, 3'-hydroxy-2'-tetrahydropyranylnoviosyloxy-3-benzamidocoumarin **6** was prepared from coumermycin A_1 by the method outlined in Scheme 1.

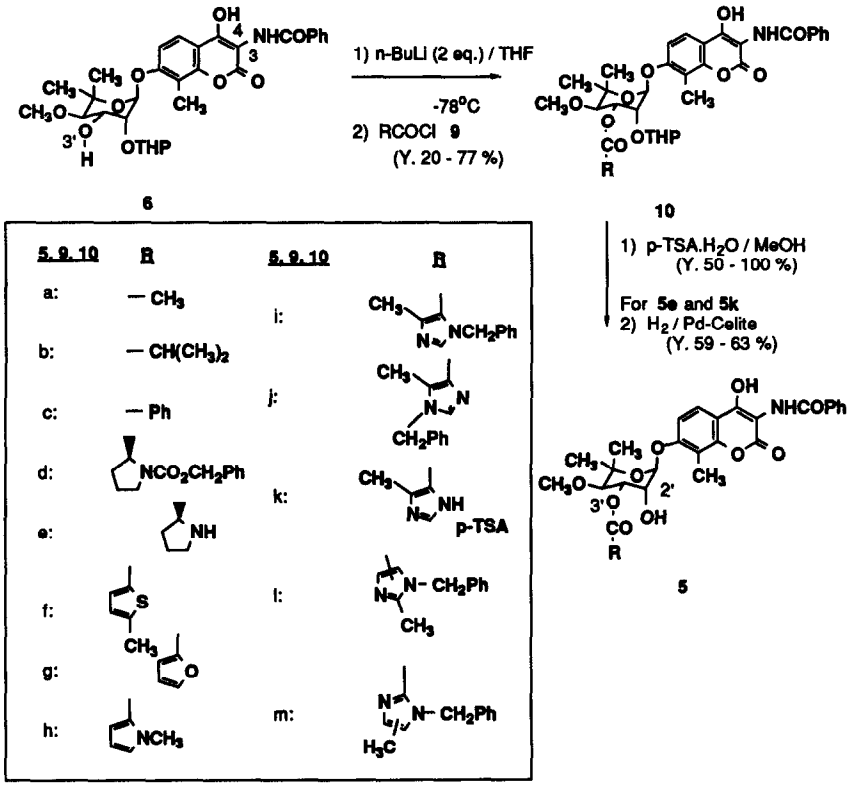
SCHEME 1



The 2'-hydroxy groups of coumermycin A_1(**1**) were first protected as the tetrahydropyranyl derivative **7**[5] which then was converted to the 3-benzamido derivative **8**[5] in 50-60% yield. The pyrrole carboxylate was cleaved by treatment with hydrazine [4a] to produce the desired 3'-hydroxy compound **6** in 74% yield.

The 3'-hydroxy group in compound **6** was acylated[6] selectively by generation of the dianion at the C-4 OH and the C-3' OH using 2 eq. of n-BuLi at -78°C in THF followed by treatment with 1 eq. of acyl chloride **9**[7] at temperatures ranging from -78°C to room temperature (Scheme 2). Using this dianion-acylation process, a number of 3'-esters **10a - 10d** and **10f-10j** were prepared in 20 - 77 % yield.[12] However, it should be noted that the imidazole acid chlorides **9l** and **9m** were resistant to reaction with the dianion generated from **6**, and as a result, the imidazole ester derivatives **10l** and **10m** could not be prepared. The tetrahydropyranyl protecting group in **10** was removed with p-toluenesulfonic acid monohydrate (p-TSA•H_2O) in MeOH to afford the target molecules **5a-5d** and **5f-5j** in 50 - 100 % yield.[10] The (L)-proline derivative **5e** and imidazole analog **5k** were prepared in 59 and 63 % yield, respectively, from the corresponding N-protected compounds **5d** and **5i** (or **5j**) by catalytic hydrogenation of the benzyloxycarbonyl group or the benzyl group.

SCHEME 2



It is interesting to note that the 3'-aliphatic carboxylate esters, such as acetate **5a** and proline ester **5e**, were found to be hydrolytically labile, being transformed to a mixture of the corresponding compound with a 3'-hydroxy and 2'-ester group (O-acyl migration) and 2',3'-dihydroxy compound **6** (2'-OH).[13] However, the 3'-phenyl and 3'-heteroaromatic carboxylate esters, **5c**, and **5f - 4k** were stable and no obvious O-acyl migration was observed.

The *in vitro* antibacterial activity of some of these new derivatives is summarized in Table 1. Compound **4**, 5-methylpyrrole-2-carboxylate derivative, is included for comparison. Staphylococcal species selected for evaluation are the methicillin-sensitive strain of *Staphylococcus aureus* (A9537), the methicillin-resistant strain of *Staphylococcus aureus* (A20700) and the methicillin-resistant strain of *Staphylococcus epidermidis* (A25441). Antibacterial activities are expressed as the minimum inhibitory concentrations (MIC's).

The 3'-aliphatic and aromatic carboxylate esters **5a-5e** were devoid of useful antibacterial activity against the staphylococcal species tested. The 3'-heteroaromatic carboxylate esters **5f-5i** and **5k** showed weak levels of anti-staphylococcal activity, with 5-methylthiophene-carboxylate **5f** being the most active analog in the series. It was more potent than 5-methylpyrrole-carboxylate **4** against the methicillin-sensitive strain of *Staphylococcus aureus* but less potent against the methicillin-resistant strains of staphylococcal species. The furan, N-methylpyrrole and basic imidazole analogs **5g, 5h, 5i** and **5k** were much less active than the compound **4**. The poor activity of these four derivatives may be a reflection of the lack of the methyl group at the proper position of the heteroaromatics. It appears at this stage, that the 5-methylpyrrole-2-carboxylate found in coumermycin A_1 and compound **4** is still the best substituent for exhibiting uniformly good anti-staphylococcal activity, including the methicillin-resistant strains.

TABLE 1: Selected *In Vitro* Antibacterial Activity of New Semi-Synthetic Noviosylcoumarins 5, MIC's (μg/mL)*

Compounds	*S. aureus* (A9537)	*S. aureus*/MR (A20700)	*S. epidermidis*/MR (A25441)
4	0.13	0.13	0.13
5f	0.03	4	8
5g	8	8	8
5h	2	8	8
5i	8	4	2
5k	16	16	8

*Determined by the 2-fold serial broth dilution method using nutrient broth, inoculum size: 5 x 10^5 cfu/mL. For methicillin-resistant strains (MR), the incubation was carried out at 35°C for 24 hours.
Abbreviations: *S. aureus* = *Staphylococcus aureus*, *S. epidermidis* = *Staphylococcus epidermidis*.

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References and Notes:

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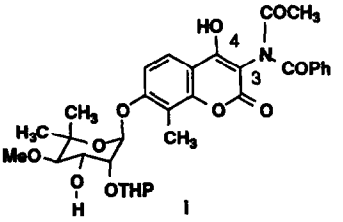
1. The term "noviosylcoumarin antibiotics" is used here to represent a class of coumarin-glycoside antibiotics such as coumermycin A_1 and novobiocin. For review on coumarin-glycoside antibiotics, see Berger, J; Batcho, A.D. In *Antibiotics:Isolation, Separation and Purification*; (J. Chromatography Library 15); Winstein, M.J., Wagmen, G.H. Eds.; Elsevier: London, **1979**; pp 101-158.
2. Rahl, J.J. In *Antimicrobial Agents Annual/1*; Peterson, P.K., Verhoof, J. Eds. Elsevier: New York, **1986**; Chapter 44, pp 489-514.
3. a) Gellert, M.; O'Dea, M.H.; Itoh, T.; Tomizana, J.-I. *Proc. Natl. Acad. Sci. USA*, **1976**, *73*, 4474.
   b) Hooper, D.C.; Wolfson, J.S.; McHugh, G.L.; Winters, M.B.; Swartz, M.N. *Antimicrob. Agents Chemother.* **1982**, 662.
   c) Rádl, S. *Pharmac. Ther.* **1990**, *48*,1.
4. a) Godfrey, J.C.; Price, K.E. *Adv. Appl. Microbiol.* **1972**, *15*, 231.
   b) Keil, J.G.; Godfrey, J.C.; Cron, M.J.; Hooper, I.R.; Nettleton, D.E.; Price, K.E.; Schmitz, H. *Pure Appl. Chem.* **1971**, *28*, 571.
   c) Ueda, Y.; Chuang, J.M.; Crast, L.B.; Partyka, R.A. *J. Antibiot.* **1989**, *42*, 1379.
5. a) Keil, J.G.; Hooper, I.R.; Cron, M.J.; Fardig, O.B.; Nettleton, D.E.; O'Herron, F.A.; Ragan, E.A.; Rousche,M.A.; Schmitz, H.; Schreiber, R.H.; Godfrey, J.C. *J. Antibiot.* **1968**, *21*, 551.
   b) Ueda, Y., Chuang, J.M.; Crast, L.B.; Partyka, R.A. *J. Org. Chem.* **1988**, *53*, 5107.
6. A conventional acylation procedure ($CH_3COCl/Et_3N/CH_2Cl_2$) produced only C-3 N-acetyl derivative **i**. This is presumably due to acylation at the C-4 phenolic hydroxy group followed by transacetylation to the N-acetate **i**.



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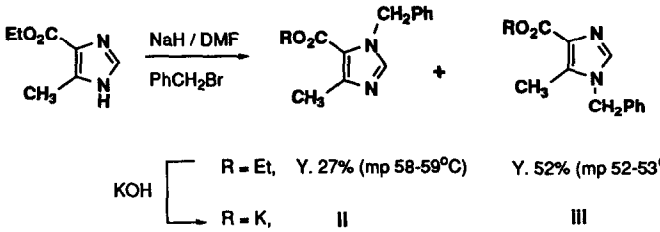
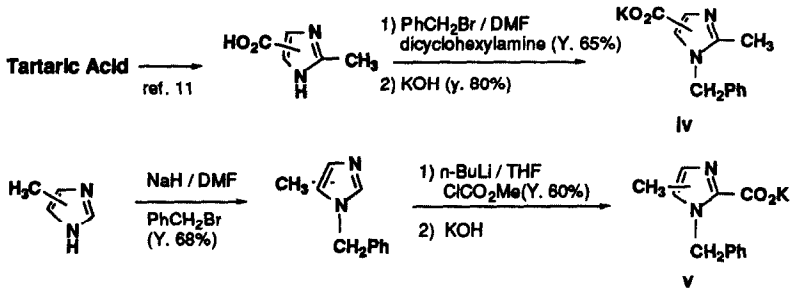
7. The acid chlorides **9f**[8] and **9h**[9] were prepared by the literature procedure. The other acid chlorides **9d**, **9i**, **9j**, **9l**, and **9m** were prepared by treatment of the corresponding carboxylic acids with $SOCl_2$-Et_3N (for **9d**, **9m**) or the potassium salts with oxalyl chloride (for **9i**, **9j**, **9l**), and these acid chlorides were used without purification.
   N(1)-Benzyl-4-methylimidazole-5-carboxylic acid (**ii**) and N(1)-benzyl-5-methylimidazole-4-carboxylic acid (**iii**), precursors of **9i** and **9j** respectively, were prepared as potassium salts from ethyl 4(5)-methylimidazole-5(4)-carboxylate by N-benzylation,[10] separation of each isomers and hydrolysis with KOH.



The other geometric isomer of N-benzylmethylimidazole carboxylic acids, N-benzyl-2-methylimidazole-4(5)-carboxylic acids (iv) and N-benzyl-4(5)-methylimidazole-2-carboxylic acids (v) were prepared as potassium salts, respectively from tartaric acid[11] and 4(5)-methylimidazole as illustrated below, and used to prepare acid chlorides **9l** and **9m**.



8. Harris, J.A.; Arthur, J.C. Jr.; Hinojosa, O.; Boudafaux, G.J.; McCall, E.R. *Carbohydrate Research* **1977**, *53*, 57.
9. Walker, G.N. *U.S. Patent* (Ciba-Geigy Corp.) 4,435,407 (Mar. 6, 1984).
10. a) Davey, D.D. *J. Org. Chem.* **1987**, *52*, 4379.
b) Bolhofer, W.A.; Cragoe, E.J.; Hoffman, J.M. *U.S. Patent* (Merck & Co., Inc.) 4,220,654 (Sep. 2, 1980)
11. Fargher, R.G.; Pyman, F.L. *J. Chem. Soc.* **1919**, *115*, 217.
12. All new compounds gave satisfactory analytical and spectroscopic results in accordance with the assigned structure.
A typical procedure (preparation of compound **5f**): To a solution of 3'-hydroxy compound **6** (114 mg, 0.2 mmol) in anhydrous THF (4 mL) was added at -78°C (dry-ice/acetone), under dry nitrogen atmophere, n-BuLi (0.26 mL, 1.58 M hexane solution, 0.4 mmol; 2 eq.) and the yellow mixture was stirred for 5 min. To this was injected 5-methylthiophene-carboxylic acid chloride **9f** (30 ml, 0.25 mmol) and the mixture stirred in dry-ice/acetone bath for 1 hr and at -50°C for another hour. The mixture was quenched with sat'd NH_4Cl, and extracted with EtOAc. The ethyl acetate extract was washed (brine), dried (Na_2SO_4), concentrated and purified by silica gel column (10% EtOAc/CH_2Cl_2) to obtain 97 mg (0.14 mmol, y. 70 %). of **10f** (diastereomeric mixture) as white crystals: mp 183-186°C (MeOH). A solution of **10f** (82 mg, 0.12 mmol) in a mixture of CH_2Cl_2 (1 mL) and MeOH (4 mL) was treated with p-toluenesulfonic acid monohydrate (10 mg, 0.053 mmol) at room temperature for 20 h and the mixture was diluted with CH_2Cl_2, washed (brine), dried (Na_2SO_4), concentrated and purified by silica gel column (50% EtOAc/CH_2Cl_2) to obtain 60 mg (0.098 mmol, y. 82%) of **5f** as white crystals: mp 199-201°C (MeOH); Rf 0.38 (50% EtOAc/CH_2Cl_2). ^1H NMR (DMSO-d_6, 300 MHz) δ ppm: 1.09 (3H, s, 5'-Me), 1.31 (3H, s, 5'-Me), 2.26 (3H, s, 8-Me), 2.54 (3H, s, 5''-Me), 3.50 (3H, s, 4'-OMe), 3.69 (1H, d, J=10 Hz, 4'-H), 4.24 (1H, m, 2'-H), 5.46 (1H, dd, J=3, 10 Hz, 3'-H), 5.62 (1H, d, J=2.5 Hz, 1'-H), 5.81 (d, J=5.5 Hz, 2'-OH; D_2O exchanged), 6.99 (1H, d, J=3.5 Hz, 4''-H), 7.21 (1H, d, J=9 Hz, 6-H), 7.5-7.6 (3H, m, Ph-H_s), 7.74 (1H, d, J=3.5 Hz, 3''-H), 7.76 (1H, d, J=9 Hz, 5-H), 8.02 (2H, d, J=7 Hz, Ph-H_s), 9.48 (s, 3-NH); IR (KBr) 3460, 3370, 1703, 1690, 1630, 1600 cm^{-1}; MS (FAB/NOBA+NaI+KI) m/e 610 (MH^+), 632 (MNa^+), 648 (MK^+); UV (EtOH) λmax 281 (ε 1.86 x 10^4), 322 nm (ε 1.59 x 10^4); Anal. calcd for $C_{31}H_{31}NO_{10}S$: C, 61.07; H, 5.13; N, 2.30; S, 5.26. Found: C, 61.03, H, 5.23; N, 2.27; S, 5.32.
13. This type of O-carbonyl group migration was observed in novobiocin.[14] A similar O-acyl migration has also been documented in other natural products (e.g. Ganefromycins[15]). The 2',3'-dihydroxy compound **6** (2'-OH) was devoid of antibacterial activity.
14. Hinman, J.W.; Caron, E.L.; Hoeksema, H. *J. Amer. Chem. Soc.* **1957**, *79*, 5321.
15. Carter, G.T.; Phillipson, D.W.; West, R.R.; Borders, D.B. *J. Org. Chem.* **1993**, *58*, 6588.

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