Isolation and Synthesis of Shark-Repelling Saponins

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ABSTRACT: Saponins are complex compounds that are composed of a saccharide attached to a steroid or triterpene. They are natural surfactants, or detergents. Several important biological effects have been ascribed to saponins. They have been isolated from a great number of terrestrial plants. In the animal kingdom they are found in most sea cucumbers and starfish, whereas they are found only rarely in alcyonarians, gorgonians, sponges, and as shark-repelling compounds in fish. The present review deals with the isolation and some syntheses of the shark-repelling saponins mosesins-1 to -5 and pavoninins-1 to -6 obtained from the fish species *Pardachirus.*

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Saponins are complex compounds that are composed of a saccharide attached to a steroid or triterpene. They are natural surfactants, or detergents. Upon shaking with water, they form colloidal solutions, giving soapy lathers. A wide range of biological effects have been ascribed to saponins, such as membrane-permeabilizing, immunostimmulant, hypocholesterolemic, and anticancer properties (1,2). They can also kill protozoa and mollusks, be used as antioxidants, cause hypoglycemia, and act as antifungal and antiviral agents (1,2). They have been isolated from a great number of terrestrial plants and are most abundant in the desert plants yucca and quillaja. Saponins are uncommon constitutents among members of the animal kingdom. They are found in nearly all sea cucumbers and starfish, whereas they are rare in alcyonarians, in gorgonians sponges, and as shark-repelling compounds in fish (3). The present review deals with the isolation and some syntheses of shark-repelling saponins obtained from fish.

Ichthyocrinotoxic fish secrete biologically active compounds that repel their predators. In 1974 the Moses sole *Pardachirus marmoratus,* which lives in the Red Sea and the western Indian Ocean, was reported to emit a toxic secretion when it was about to be bitten (4). The structures of the shark-repelling compounds were shown to be the five steroidal saponins called mosesins-1 to -5 (**1–5**) (5). The mosesins have the β-bond of C-1 of the monosaccharide galactose attached at the 7α position of the (25)-3,7α,26-trihydroxycholestane 26-acetate skeleton with varying substitution and oxidation at C-3,4,5,6,12α and -15α on the steroid (**1–5**), as shown in Figure 1.

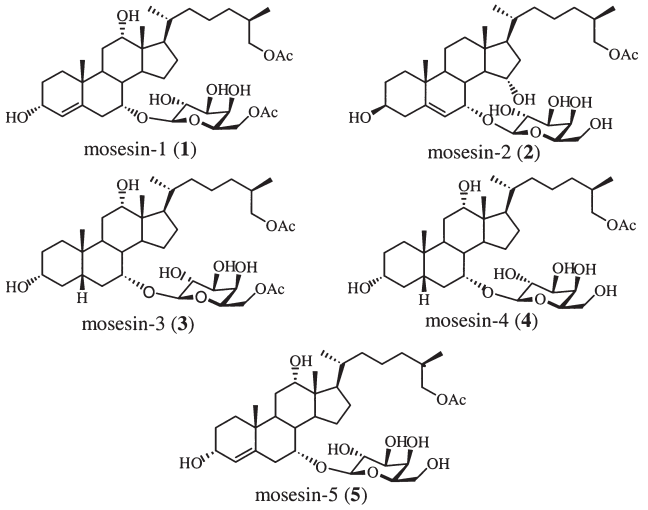

FIG. 1. Mosesins 1–5 (**1–5**).

The synthesis of mosesin-4 (**4**) is outlined in Scheme 1 (6). Cholic acid (**6a**) was used as the starting material since it has the hydroxy groups 3α, 7α, and 12α together with a *cis*-A,B ring junction. To synthesize **4**, the 26-hydroxy cholesterol side chain and the sugar need to be attached. The side chain was synthesized by photochemical decarboxylation/iodination of the cholic ester **6b** to yield the iodide **7a**. Conversion of the iodide **7a** to the alcohol **7b** followed by oxidation gave the aldehyde **8**. Wittig reaction of **8** with the appropriately substituted benzyl ether gave a mixture of 23*E* and *Z* isomers, **9**. The acetates were hydrolyzed, and the more reactive C-3α alcohol was protected as its ethyl carbonate **10**. The sugar was attached by reacting the steroidal diol **10** with β-galactose peracetate, using trimethylsilyl triflate as a promotor in 1,2-dichloroethane to yield the glycoside **11a** in 40% yield. To selectively introduce the C-26 acetate, the acetates in **11a** were hydrolyzed and replaced with silyl ethers, **12**. Catalytic reduction simultaneously reduced the double bond in the side chain and cleaved the benzyl ether. Acetylation at C-26 followed by cleaving the silyl ethers with hydrogen fluoride afforded mosesin-4 (**4**).

In 1984, six shark-repelling saponins, pavoninin-1 to -6 (**13–18**), were isolated from a related species of fish, *P. pavoninus,* living in the tropical regions of the western

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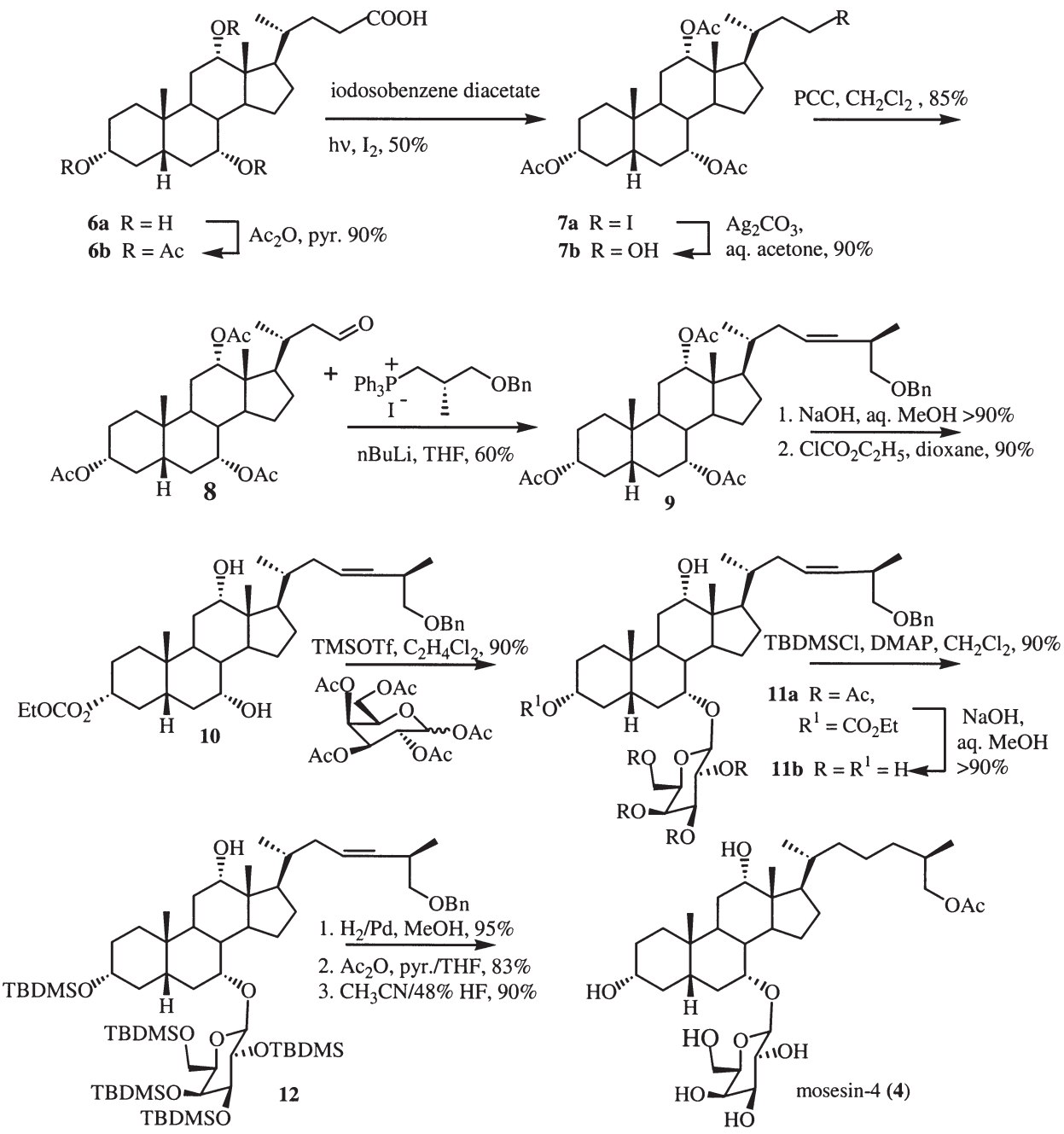
Abbreviations: LAH, lithium aluminum hydride; MEMCl, methoxyethoxymethyl chloride.



SCHEME 1. Synthesis of mosesin-4 (**4**) from cholic acid (**6a**).

Pacific and eastern Indian Oceans (7,8). The pavoninins have the β-bond of C-1 of *N*-acetylglucosamine attached at 7α or 15α to a (25*R*)-26-hydroxy- or -26-acetoxycholestane skeleton with varying oxidation at C-3,4,5,6, and -7 (**13–18**), as shown in Figure 2.

The first synthesis of the aglycones of pavoninin-1 and -2 (**28** and **27**, respectively), which contain 7α hydroxylation, was reported in 1997 and is outlined in Scheme 2 (9). 26-Hydroxycholesterol **21** was chosen as the starting material since it had been previously prepared by Clemmensen reduction of diosgenin **19** followed by C-16 deoxygenation of the resulting 3α,16β,26-triol **20** (10). Selective silylation of **21** at C-26 followed by Oppenauer oxidation of the C-3 alcohol in **22** and acetylation of the desilylated C-26 alcohol afforded the enone acetate **23**. The C-7α alcohol was prepared by lithium aluminum hydride (LAH) reduction of the C-6α epoxide **25**. The epoxide **25** was made by *meta*-chloroperoxybenzoic acid oxidation of the 4,6-dien-3-one **24**, itself obtained by 2,3-dichloro-5,6-dicycano-1,4-benzoquinone oxidation of the enone **23**. The aglycone of pavoninin-2, **27**, was prepared by treatment of the triol **26** with manganese dioxide. Selective acetylation of **27** afforded the aglycone of pavoninin-1, **28**.

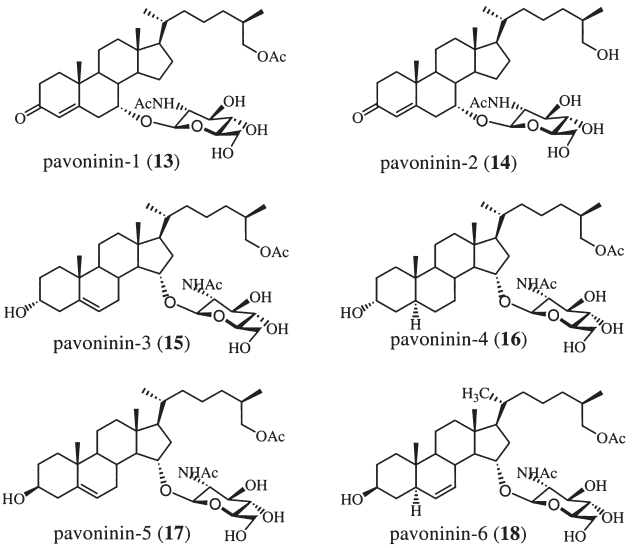
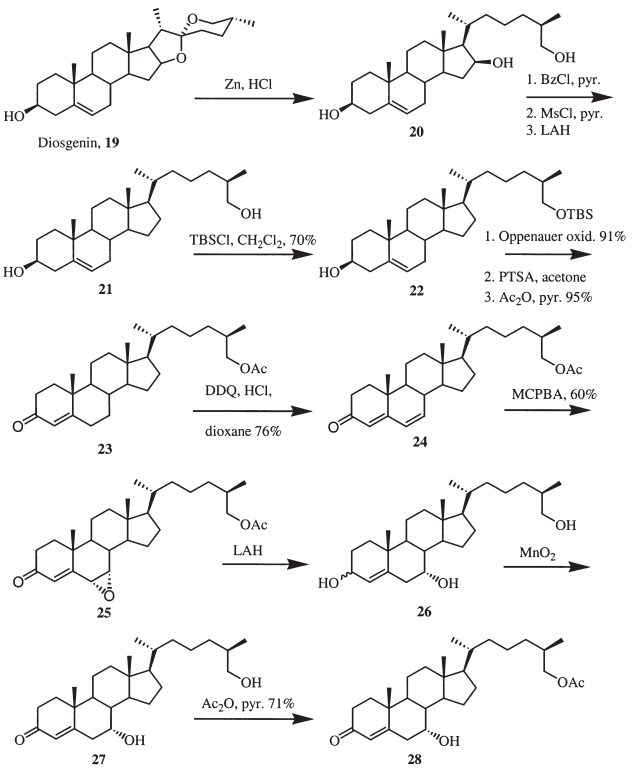
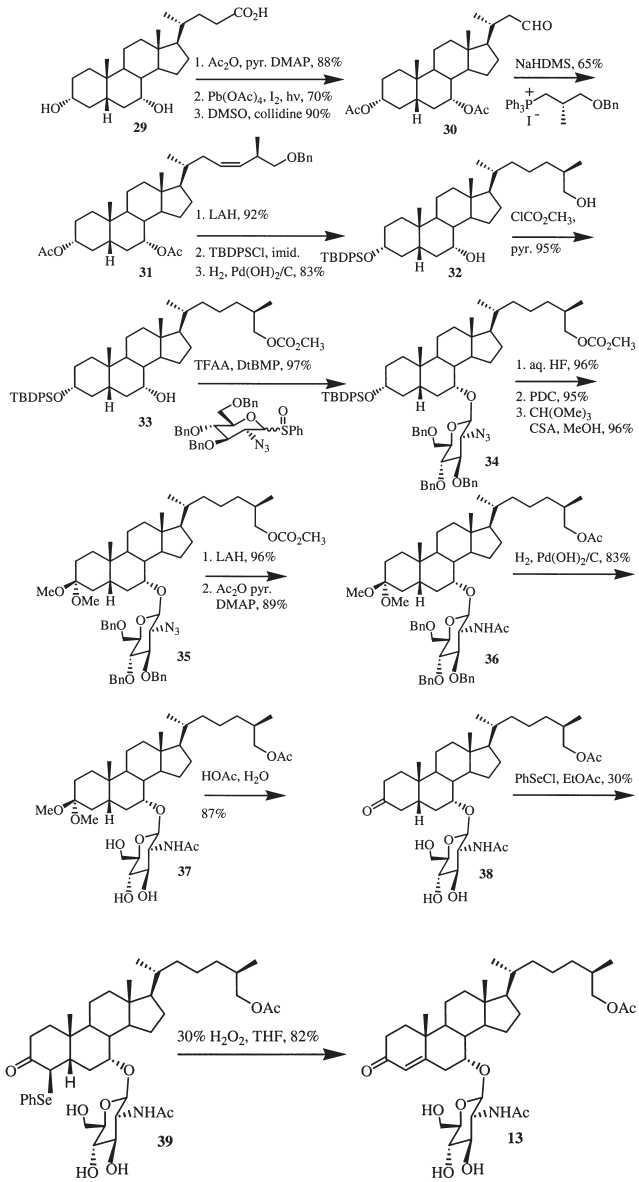


FIG. 2. Pavoninins 1–6 (**13–18**).



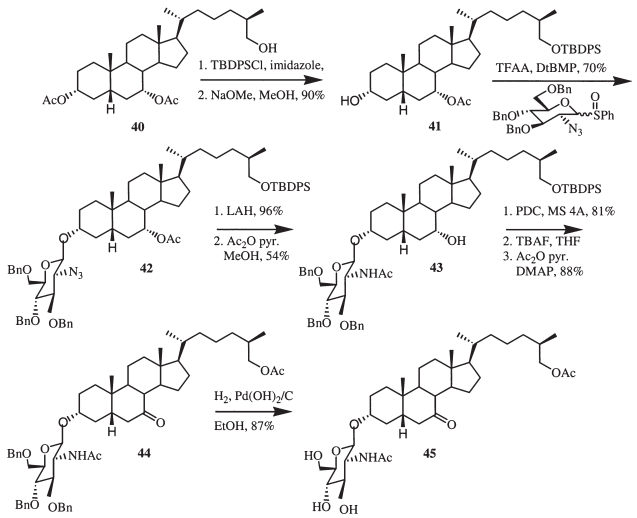
SCHEME 2. Synthesis of the aglycones of pavoninin-1 and -2 (**28** and **27**).

In the same year (1997), the first total synthesis of the saponin pavoninin-1 (**13**) was reported (11) and is outlined in Scheme 3. The synthesis of the aglycone started with commercially available chenodeoxycholic acid **29** being suitably oxidized at C-3 and C-7α. The side chain was elongated following the method used for mosesin-4, with some modifications. The aldehyde **30** was synthesized from the intermediate iodide by oxidation with DMSO and collidine. Wittig addition generated the *Z*-olefin **31**. Reductive cleavage of the acetates in **31** with LAH furnished a diol that was selectively protected at C-3. Catalytic hydrogenation simultaneously reduced the olefin and hydrogenolyzed the benzyl ether to yield the 7α,26-diol **32**. Reprotection of the C-26 alcohol as a methyl carbonate afforded the 7α alcohol **33**. Glycosylation of the hindered C-7α alcohol in **33** using a glycosyl sulfoxide gave the saponin **34**. Treatment with hydrogen fluoride liberated the C-3 alcohol, which was oxidized to the ketone with pyridinium dichromate and protected as its dimethoxy ether, **35**. Reduction of the azide group in **35** and removal of the methoxycarbonyl group with LAH followed by acetylation afforded the saponin **36**. The benzyl protecting groups on



SCHEME 3. Synthesis of pavoninin-1 (**13**).

the sugar were removed by catalytic reduction to yield **37**. Hydrolysis of the ketal gave the dihydropavoninin-1 **38**. Treatment of **38** with phenyl selenyl chloride followed by hydrogen peroxide oxidation afforded pavoninin-1 (**13**) *via* the intermediate phenyl selenide **39**.

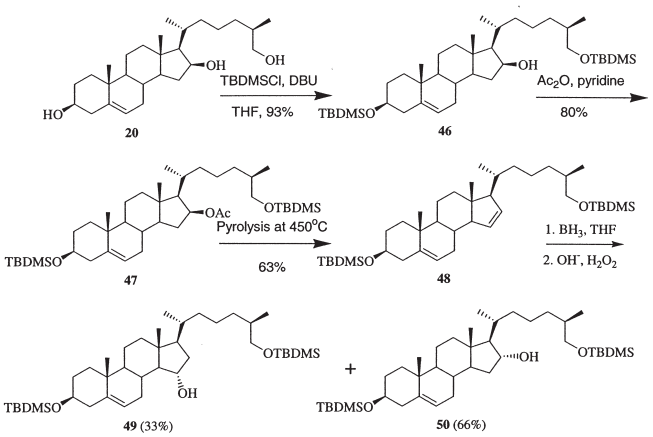


SCHEME 4. Synthesis of a structural analog of pavoninin-1 (**45**).

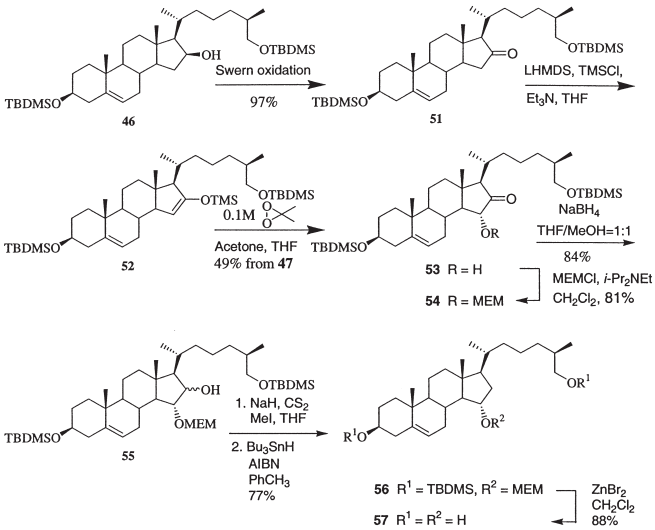
To test the structural requirements for biological activity, the analog **45** was synthesized as outlined in Scheme 4 (11). In this structure the C-3 and C-7 functionalities have been reversed. The synthesis of **45** started with catalytic reduction of the *Z*-olefin **31** prior to deacetylation and proceeded with simultaneous hydrogenolysis of the benzyl ether to yield the hydroxy diacetate **40**. Reprotection of the C-26 alcohol as its *tert*-butyldiphenylsilyl ether and selective hydrolysis of the C-3 acetate gave the 3α alcohol **41**. Glycosylation of **41** gave the glycoside **42**. LAH reduction and acetylation gave the saponin **43**. Oxidation of the C-7 alcohol and desilylation at C-26 followed by acetylation gave the acetate **44**. Hydrogenolysis of the benzyl ethers by catalytic reduction gave the structural analog **45**.

The ichthyotoxicity of pavoninin-1 **13**, the structural analog **45**, and the dihydropavoninin-1 intermediate **38** have been compared previously (11). The results showed that dihydropavoninin-1, **38**, was lethal to Japanese killifish at twice the concentration of pavoninin-1 **13**, showing it is less toxic. By contrast, twice the concentration of **45** was nontoxic to killifish. Thus, it appears that the saponins that are structurally unique, i.e., having the glycoside orthogonal to the steroid skeleton, have ichthyotoxicity activity. The structural analog **45**, which parallels the usual saponin structure, has significantly less or no ichthyotoxicity activity.

Pavoninins-3 to -6 (**15–18**) all have the sugar attached at C-15α on a cholesterol skeleton (7,8). A logical starting material for the synthesis of the C-15α pavoninins, as outlined in Scheme 5, is the commercially available diosgenin, **19**,which has functionality in positions suitable for conversion to the C-15α pavoninins. Recent modification of the Clemmensen reduction with the removal of mercury has resulted in a significant improvement in the yield of 3β,16β,26-triol, **20** (12). Dehydration of the 3,26-diprotected-16β-alcohol **46**, *via* a *syn* elimination of the 16β acetate **47**, afforded the C-15 olefin **48**. Chemo- and regioselective hydroxylation from the less hindered α face should yield the desired C-15α alcohol **49**. However, attempts to hydroxylate **48** regioselectively using substituted alkyl boranes were unsuccessful, as there was no reaction. When diborane followed by basic hydrogen peroxide oxidation was used, a mixture of C-15α and C-16α hydroxy steroids, **49** and **50**, was formed in the ratio of 1:2, with the undesired C-16α hydroxy steroid **50** as the major component.



SCHEME 5. Hydroboration–oxidation of 3,26-disilyoxycholesta-5,15-diene (**48**).



SCHEME 6. Synthesis of 26-*O*-deacetyl pavoninin-5 aglycone (**57**).

Another approach for the synthesis of the aglycone of 26-*O*-deacetyl pavonin-5, **57**, is outlined in Scheme 6 (13,14). The initial plan was to oxidize the C-16 alcohol in **46** to a ketone, introduce the C-15α hydroxyl, and then remove the ketone. Swern oxidation of 3,26-bissilyl ether **46** gave the C-15

ketone **51**. Because of the difficulty of direct hydroxylation of **51**, the ketone was hydroxylated through its silyl enol ether. The silyl enol ether, **52**, was synthesized by adding lithium hexamethyldisilazane to the solution of **51** and trimethylsilyl chloride, followed by triethylamine, then quenching the reaction with saturated $NaHCO_3$. The silyl enol ether, **52**, was not purified because it is sensitive to silica gel. Oxidation of **52** with dimethyldioxirane, followed by decomposition of the unstable intermediate C-15α,16α-epoxide under mildly acidic conditions, afforded the C-15α-hydroxy ketone **53**.

The next step was the deoxygenation of the 16-ketone. However, all methods used to deoxygenate the α-hydroxy ketone using Raney nickel on the dithioketal or Wolff–Kishner reduction failed. To remove the ketone, the carbonyl was reduced to a hydroxyl group, followed by deoxygenation using Barton's reaction. Since both C-15 and C-16 would be hydroxyl groups, a different protecting group for the hydroxyl group in the 15α-position was used. Protection of the C-15α alcohol in **53** with methoxyethoxymethyl chloride (MEMCl) gave the MEMCl ether **54**. Reduction of the C-16 ketone in **54** using sodium borohydride gave **55**, a mixture of epimeric alcohols at C-16. Deoxygenation of the alcohols, **55**, *via* the xanthate using the Barton reaction yielded the MEMCl ether, **56**. Treatment of the MEMCl ether, **56**, using dry zinc bromide in dry methylene chloride cleaved the MEMCl group as well as the *tert*-butyldimethylsilyl groups to yield the target 26-*O*-deacetyl pavoninin-5 aglycone, **57**.

There were 53 unprovoked shark attacks in the United States in 2000, with approximately a third being fatal. If one could develop a compound with shark-repelling properties, when applied appropriately, the risk of shark attacks could be greatly reduced. Fish belonging to the species *Pardachirus* are known to excrete saponins that have shark-repelling properties. These natural saponins, i.e., mosesins 1–5 (**1–5**) and pavoninins-1 to -6 (**13–18**), could act as lead compounds for the synthesis of more effective shark repellents. From the results thus far, two conclusions can be drawn: (i) Saponins with shark-repelling properties appear to have the saccharide toward the middle of the steroid and orthogonal to its axis. (ii) The monosaccharide can be varied, since both galactose and *N*-acetylglucosamine are both shark repellents when attached to the C-7α of (25*R*)-26-cholesterol. Because glucose is the most abundant, most widespread, and least expensive monosaccharide, the question is, does glucose work? That is to say, would a 7α glycoside of (25*R*)-26-hydroxycholesterol be a shark repellent? It is an obvious target for synthesis.

ACKNOWLEDGMENTS

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REFERENCES

1. Francis, G., Kerem, Z., Harinder, P.S., and Becker, K. (2002) The Biological Action of Saponins in Animal Systems: A Review, *Br. J. Nutr. 88,* 587–605.
2. Press, J.B., Reynolds, R.C., May, R.D., and Marciani, D.J. (2000) Structure/Function Relationships of Immunostimulating Saponins, *Stud. Nat. Prod. Chem. 24,* 131–174.
3. Minale, L., Ricco, R., and Zollo, F. (1993) Steroidal Oligoglycosides and Polyhydroxysteroids from Echinoderms, in *Progress in the Chemistry of Organic Natural Products* (Herz, W., Kirby, G.W., Moore, R.W., Steglich, W., and Tamm, Ch., eds.), Vol. 62, pp. 75–308, Springer-Verlag, New York.
4. Clark, E. (1974) The Red Sea's Sharkproof Fish, *Natl. Geographic 146,* 718–727.
5. Tachibana, K., and Gruber, S.H. (1988) Shark Repellent Lipophilic Constituentes in the Defense Secretion of the Moses Sole (*Pardachirus marmoratus*), *Toxicon 26,* 839–853.
6. Gariulo, D., Blizzard, T.A., and Nakanishi, K. (1989) Synthesis of Mosesin-4, a Naturally Occurring Steroid Saponin with Shark Repellent Activity, and Its Analog 7β-Galactosyl Ethyl Cholate, *Tetrahedron 45,* 5423–5432.
7. Tachibana, K., Sakaitani, M., and Nakanishi, K. (1984) Pavoninins: Shark-Repelling Ichthyotoxins from the Defense Secretion of the Pacific Sole, *Science 226*, 703–705.
8. Tachibana, K., Sakaitani, M., and Nakanishi, K. (1985) Pavoninins, Shark-Repelling and Ichthyotoxic Steroid *N*-Acetylglucosaminides from the Defense Secretion of the Sole *Pardachirus pavoninus* (Soleidae), *Tetrahedron 41,* 1027–1037.
9. Kim, H.S., Kim, I.C., and Lee, S.O. (1997) Synthesis of Two Marine Natural Products: The Aglycones of Pavoninin-1 and -2, *Tetrahedron 53,* 8129–8136.
10. Noam, M., Tamir, I., Breuer, E., and Mechoulam, R. (1981) Conversion of Ruscogenin into 1α- and 1β-Hydroxycholesterol Derivatives, *Tetrahedron 37,* 597–604.
11. Ohnishi, Y., and Tachibana, K. (1997) Synthesis of Pavoninin-1, a Shark Repellent Substance, and Its Structural Analogues Toward Mechanistic Studies on Their Membrane Perturbation, *Bioorg. Med. Chem. 5,* 2251–2265.
12. Williams, J.R., Chai, D., and Wright, D. (2002) Synthesis of (25*R*)-26-Hydroxycholesterol, *Steroids 67,* 1041–1044.
13. Williams, J.R., Chai, D., Gong, H., Zhao, W., and Wright, D. (2002) Studies Toward the Synthesis of the Shark Repellent Pavoninin-5, *Lipids 37,* 1193–1195.
14. Williams, J.R., Chai, D., Bloxton, J.D., II, Gong, H., and Solvibile, W.R. (2003) Synthesis of the Aglycone of 26-*O*-Deacetyl Pavoninin-5, *Tetrahedron 59,* 3183–3188.

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