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CONFORMATIONALLY CONSTRAINED ANALOGUES OF DIACYLGLYCEROL (DAG). 4. INTERACTION OF α-ALKYLIDENE- γ-LACTONES WITH PROTEIN KINASE C (PK-C)

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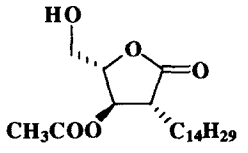
Abstract. Isomeric α-alkylidene-γ-lactones with a C_{14} chain were synthesized and evaluated as inhibitors of phorbol ester binding to protein kinase C (PK-C). While the Z-isomer **3** was the most potent inhibitor (K_i = 0.2 μM), the stereochemistry at the α-position of the lactone appears to have only a modest effect in modulating PK-C binding. The same appears to be true for both isomeric α-alkyl-γ-lactones which were obtained by catalytic hydrogenation of the unsaturated lactones.

From the structure-activity study reported in the preceding communication,[1] we found that (2R,3R,4S)-3-O-acetyl-2-alkyl-2-deoxyribonolactones bearing a long alkyl substituent (e.g. compound **1**) behaved as powerful inhibitors of [20-^{3}H]phorbol-12,13-dibutyrate (PDBU) binding to protein kinase C (PK-C). Furthermore, we were able to show that PK-C displayed a high level of discrimination between these compounds and their optical antipodes, as would be expected for a binding mechanism akin to that of the natural agonist diacylglycerol (DAG).[2] As an extension of the same investigation, we decided to explore the relationship between the particular stereochemistry at the point of insertion of the alkyl side chain (R or S) and the ability of 3R,4S-deoxyribonolactones to bind PK-C.



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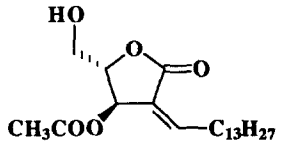

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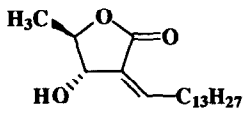

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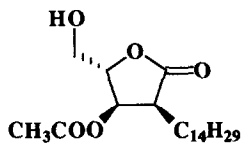

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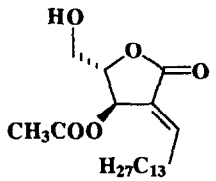

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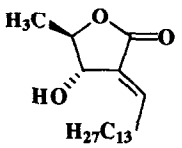

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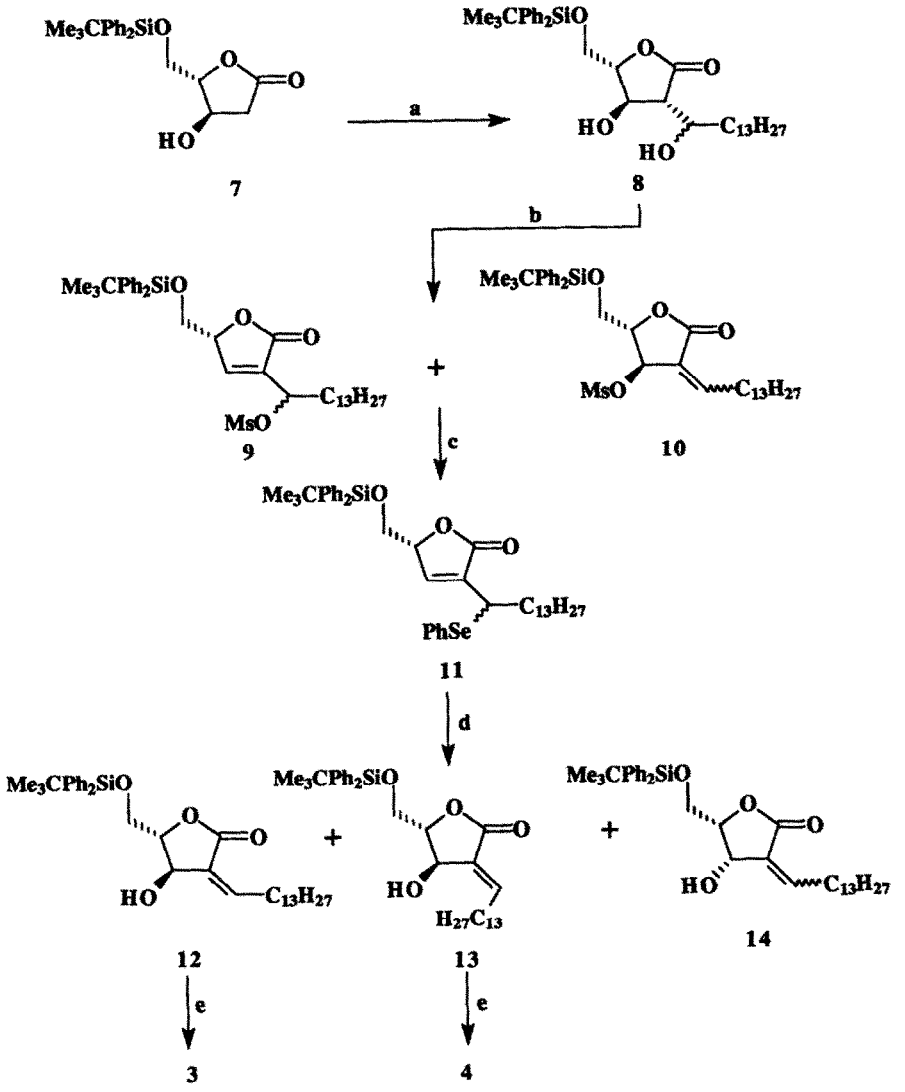
This investigation was considered important because a change in the orientation of the C-2 alkyl

chain would affect 1) the efficiency of anchoring and insertion of the molecule into the membrane bilayer and 2) the orientation of the polar pharmacophores at or near the bilayer interface. Having established previously that a C_{14} carbon chain was more effective than a C_{12},[1] additional targets (**2-4**) were designed in order to explore all possible chain orientations from the C-2 position of the lactone.

Since the stereoselective alkylation of the β-hydroxy lactones prepared previously yielded a C-2 alkyl chain with the R configuration,[1] we were interested in a synthetic route for obtaining the compound with the inverted S configuration. A simple approach considered to obtain this compound was from the catalytic hydrogenation of the corresponding α-alkylidene-γ-lactones (**3** or **4**) from which both **2** and its known diastereoisomer **1**[1] were expected to be isolated. This approach was additionally attractive because the isomeric precursors would, themselves, be informative targets. The *E*- and *Z*-geometries of the α-alkylidene-γ-lactones provide compounds in which the main axis of the side chain is coplanar with the α,β-unsaturated lactone ring. Furthermore, because of the rigidity of the double bond, each geometric isomer projects the side chain in a different direction within the same plane.

For this task, the procedure of Jouillié reported for the synthesis of (-)-Litsenolides C_1 (**5**) and C_2 (**6**) was adapted.[3] Litsenolides resemble the desired α-alkylidene-γ-lactones, but have the opposite 3S,4R configuration and lack the C-5 hydroxyl group.[4] As shown in the Scheme, reaction of the dilithium enolate of **7** with myristyl aldehyde afforded the expected diastereomeric mixture of diols (**8**) which on subsequent treatment with methanesulfonyl chloride gave a mixture of isomeric mesylates (**9** and **10**). This mixture was converted to compound **11** after treatment with sodium phenylselenide in the manner reported by Jouillié.[3] However, rearrangement of the allylic selenoxide of **11** —formed after treatment with hydrogen peroxide— followed a different stereochemical course due to the influence of the bulky diphenyl-*t*-butylsilyl protecting group. The reaction produced a mixture of allylic alcohols with the R configuration at C-3 favored by an almost 2:1 ratio. The geometric *E*- and *Z*-isomers **12** and **13** were separated from each other and from the geometric isomers of the C-3 epimer (**14**) by column chromatography. Acetylation and removal of the *t*-butyldiphenylsilyl group produced the target compounds **3** and **4**. The geometry of the exocyclic double bond in these compounds was assigned by ^{1}H NMR spectroscopy. For a cisoid enone system it is known that the β-*cis* vinyl proton resonates 0.3 to 0.9 ppm downfield from that of the corresponding β–*trans* proton.[4,5] The values for these protons in compound **3** (dt, δ = 6.7, J = 7.8 and 1.2 Hz) and compound **4** (dt, δ = 7.1, J = 7.9 and 1.8 Hz) are in excellent agreement with the values reported for litsenolides C_1 and C_2 (**5** and **6**).[4] During catalytic hydrogenation of either geometric isomer, facile β-elimination of the acetate group occurred which after further reduction gave a mixture of 2-alkyl-3-deoxy lactones. When the reaction was performed in non-polar solvents a mixture of isomers **1** and **2** could be separated from the 3-deoxylactones. However, separation of **1** and **2** was not possible and we were only able to obtain a chromatographic fraction that was enriched in isomer **2** (**2**:**1** = 1.4). This fraction was used for PK-C binding studies in comparison with compounds **1**, **3** and **4**. According to the ^{1}H NMR spectrum of this mixture, one of the components was identical to authentic compound **1**, which was obtained independently from the direct alkylation of the 3R,4S-lactone.[1] This

Scheme



Reagents: (a) i. LDA, THF, -78 °C ii $C_{13}H_{27}CHO$, $ZnCl_2$ (84%) (b) MsCl, Et_3N, CH_2Cl_2, –10 °C (91%) (c) $(PhSe)_2$, $NaBH_4$, EtOH, –20 °C (92%) (d) 30% H_2O_2, CH_3CN, –20 °C (**12**+**13**, 50%) (e) i. Ac_2O, pyridine, CH_2Cl_2, rt (78%) ii. HF-pyridine, THF, 0 °C (92%)

result helped to confirm that the stereochemistry at C-3 following rearrangement of the allylic selenoxide was indeed R (*vide supra*).

The biological results obtained (Table) indicate that in terms of PK-C binding, compounds with side chains with either R or S stereochemistry are associated with nearly identical potencies (compare **1** with the enriched **2**:**1** mixture). In addition, despite a three-fold increase in binding, the difference between the *Z*- and *E*-geometries of the 2-alkylidene lactones appears to be small (compare **3** and **4**).

Table. Apparent K_i Values for the Inhibition of PDBU Binding to PK-C

	PK-C (mixture)	**PK-C (α)**
cmpd	$K_i \pm$ sem (μM)	$K_i \pm$ sem (μM)
1[1]	0.6 ± 0.0 (n = 2)	0.5 ± 0.0 (n = 3)
2:**1** = 1.4	n.d.	0.6 ± 0.0 (n = 3)
3	0.2 ± 0.0 (n = 2)	0.2 ± 0.0 (n = 3)
4	0.6 ± 0.0 (n = 2)	0.3 ± 0.0 (n = 3)

When molecular models of these compounds (**1**-**4**) were examined, it was easy to see that in all cases the side chains were oriented in the same general direction, while the polar pharmacophores remained at nearly equivalent positions. It is noteworthy that the alkylidene lactone **3** is a slightly more potent in displacing labeled PDBU from PK-C than the comparable DAG analogue, glycerol-1-myristate acetate. This compound represents a useful template upon which one can incorporate additional restrictions in order to increase affinity towards PK-C.

References and Notes

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