version of **2** to **3** to give one estimate of the enantiomeric excess for **3**. Turnover numbers for the reduction (molecules of **2** reduced per atom of rhodium in 48 h) were also calculated from these conversions. The mixture of **2, 3,** and buffer was concentrated to a paste. The **2** and **3** were extracted into methanol, and converted to methyl esters with diazomethane. Examination of the NMR spectrum of this mixture in the presence of the chiral europium shift reagent $Eu(hfc)_3$[9] provided a second estimate of the enantiomeric excess for **3**. Values from optical rotation and NMR were in good agreement. Hydrogenations carried out in the absence of avidin, and in the presence of other proteins, were conducted and assayed by analogous procedures. Results are summarized in Table I. For comparison, this table also lists turnover numbers for conversion of the less hindered substrate allyl alcohol (**4**) to 1-propanol (**5**).

Compound **1·RhNBD+Tf−**, by itself, was a moderately active hydrogenation catalyst which shows no enantioselectivity in production of **3**. The presence of lysozyme, bovine serum albumin (BSA), and carbonic anhydrase (CA) in solutions of **1·RhNBD+Tf−** had no significant influence on enantioselectivity, although BSA and CA markedly lowered the activity of the catalyst (CA by approximately a factor of 10). The presence of 1 equiv of avidin in solution (assuming each avidin subunit to be associated with 1 equiv of **1**) resulted in a definite *increase* in activity, and in the production of **3** with ~40% *S* enantiomeric excess. When the ability of avidin to bind **1** was blocked by prior exposure to either a 10% excess or a tenfold excess of biotin, the enantioselectivity of the reduction was eliminated. Unexpectedly, solutions containing 1.0 equiv of **1·RhNBD+** per avidin subunit showed significantly higher enantioselectivity than those containing 0.5 equiv. The origin of this difference is not evident, but may reflect interaction between the biotin binding sites in different subunits.[3,4] The reduction in enantioselectivity observed on addition of **1·RhNBD+** to a mixture of avidin and BSA may indicate either slow dissociation of **1·Rh+** from a complex with BSA or interaction between BSA and avidin·**1·Rh+**.

The observations summarized in Table I are compatible with the hypothesis that the active catalyst in solutions of **1·RhNBD+Tf−** and avidin is a complex in which **1** is associated with the protein at the biotin-binding site. The observation that the turnover numbers for **2 → 3** and **4 → 5** are roughly parallel suggests little gross structure sensitivity to the system. The catalyst system composed of **1·Rh(I)** bound to avidin is not a practical asymmetric catalyst: although avidin is commercially available, it is expensive by the standards of transition metal catalysis; the enantioselectivity displayed by avidin·**1·Rh(I)** in hydrogenation of **2** to **3** is only modest.[7] Nonetheless, the experiments summarized here establish two principles. First, it is possible to carry out homogeneous hydrogenation using a diphosphinerhodium(I) catalyst associated with a protein: neither the aqueous solution nor interactions between the metal and the protein necessarily deactivate the catalyst. Second, the chirality of the protein is capable of inducing significant enantioselectivity in the reduction. It may be possible to apply these principles to the development of other combinations of proteins and transition metals capable of effecting practical enantio- or regioselective hydrogenation.[10] Further, the techniques developed to bind transition metals to specific sites in proteins may find uses in biological and clinical chemistry unrelated to asymmetric synthesis. We will describe further studies in this area in subsequent publications.

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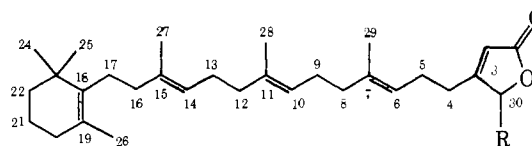
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Mokupalides, Three Novel Hexaprenoids from a Marine Sponge[1]

Sir:

Marine sponges, the most primitive multicellular invertebrate animals, have become recognized for their synthetic virtuosity, which approaches that of microorganisms and which has within the past few years revealed a stunning spectrum of new organic structures.[2] In our continuing search for physiologically active marine metabolites we have isolated from a dark green sponge[3] collected at Enewetak atoll in the Marshall islands three hexaprenoids, which represent a new type of C_{30} isoprenoids and to which we have assigned structures 1–3.

1, R = OH; 2, R = OAc; 3, R = H

Extraction of the freeze-dried sponge (petroleum ether, Soxhlet) followed by chromatography (Bio-Sil A, hexane with increasing EtOAc from 2%), which was monitored by ¹H NMR, yielded with 7% EtOAc in hexane **2** (0.255 g), **3** (0.678 g), and with 12% EtOAc **1** (1.67 g). Steroids (0.707 g) were eluted between **3** and **1**. Compound **1**, which we have named hydroxymokupalide,[4] was further purified on Sephadex LH-20 (CH_2Cl_2/hexane, 4:1) and once more on Bio-Sil A (hexane/EtOAc) to a colorless syrup (1.05 g, 0.). homogeneous on TLC (R_F 0.14, hexane/EtOAc, 4:1), $C_{30}H_{46}O_3$ (454.34455, calcd 454.34470).[5]
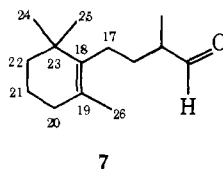
The C_{30} formula and six ¹H NMR signals assigned to methyls, one *gem*-dimethyl on a quaternary carbon and four olefinic methyls, suggested isoprenoid character. Successive mass spectral peaks at 341 ($M^+ − C_5H_5O_3$), 273 ($341 − C_5H_8$), 205 ($273 − C_5H_8$), and 137 ($205 − C_5H_8$) strengthened this hypothesis and pointed to a structure embracing

Table I. NMR Data for the Mokupalides

Compd	C-1	C-2	C-3	C-30	H-2	H-30
1	172.1	117.1	169.9	99.3	5.59	5.69
2	169.6	118.3	166.6	93.8	5.60	6.70
3	173.7	115.4	169.9	72.9	5.49[a]	3.90[b]

[a] t (*J* = 2 Hz). [b] 2 H, d (*J* = 2 Hz).

Table II. Carbon Chemical Shift Comparison of the Mokupalides with **7**

7

Compd	C-17	C-18	C-19	C-20	C-21	C-22	C-23	C-24,25	C-26
1	25.1	135.7	126.6	32.6	19.5	39.6	34.8	28.5	19.7
2	25.0	135.7	126.6	32.7	19.5	39.5	34.9	28.5	19.7
3	25.6	135.7	126.6	32.7	19.5	39.5	34.9	28.6	19.6
7	25.8	136.3	127.0	32.6	19.3	39.6	34.7	28.4	19.6

cyclic $C_{10}H_{17}$ (m/e 137) and $C_5H_5O_3$ (m/e 341, $C_{25}H_{41}$) termini connected by three isoprenes. The functionalized terminus of the major constituent (**1**), which is the ring tautomer of a β-alkyl-β-formylacrylic acid (maleic acid semialdehyde), was elucidated by spectral analysis and by conversion to **2** and **3**, and by ozonation of **3**. Infrared bands at 3570, 1780 (sh), and 1765 cm^{-1} assigned to hydroxyl and to an α,β-unsaturated γ-lactone possessing α-H[6] accounted for all three oxygens. A UV maximum at 211 nm (17 300) agreed with this assignment.[7] Addition of base produced a second UV band at 251 nm (4800), reversible with acid, and indicative of the hemiacetal–aldehyde relationship of this moiety. ^{1}H and ^{13}C NMR data[8] (Table I) provide further corroborative evidence.

Acetylation of **1** (pyridine, Ac_2O overnight, 2 °C) yielded acetoxymokupalide (**2**) identical (TLC, ^{1}H NMR and mass spectra) with **2** as isolated from the animal and further purified on Sephadex LH-20 (CH_2Cl_2/hexane, 4:1) to a pale yellow mobile oil: $C_{32}H_{48}O_4$ (496.35513, calcd 496.35527); λ_{max}^{MeOH} 210 nm (17 200); $\lambda_{max}^{MeOH/OH^-}$ 210 (18 800), 251 (4300); ν_{max} 1770 cm^{-1} (vbr); δ 2.23 (3 H, s).

Reduction ($NaBH_4$, MeOH, 30 min) of **1** furnished mokupalide (**3**) as a colorless oil identical with the natural product after further purification by Sephadex LH-20 (CH_2Cl_2/hexane, 4:1) and Bio-Sil A (hexane/EtOAc, 9:1): $C_{30}H_{46}O_2$ (438.35114, calcd 438.34979); λ_{max}^{MeOH} 213 (18 300) unchanged by acid or base; ν_{max} 1780, 1750, 1640 cm^{-1}.

Mokupalide (**3**) was ozonized (hexane/EtOAc, 2:1, −40 °C, 15 min) with reductive (H_2, Pd/C) workup, yielding three major products, 6,6-dimethylundecane-2,5,10-trione (**4**, TLC,

4

5   **6**

1:1 hexane/EtOAc, R_F 0.024), 4-oxopentanal (**5**, R_F 0.06), and 5-hydroxy-4-oxopentanal (**6**, R_F 0.02). About half of the ozonolysis product was further purified by Sephadex LH-20 chromatography (chloroform/MeOH, 4:3). Structures of all three fragments are based on consistent (including high resolution) mass spectra and ^{1}H NMR data (see formula, δ units) for **4**. Compound **4** establishes 13 carbons of the hydrocarbon terminus of the mokupalides while **6** provides additional evidence for the lactone terminus. The other half of the ozon-

olysis product after $NaBH_4$ in MeOH, benzoylation, and LH-20 chromatography (hexane/EtOAc, 4:1) led to 1,4-dibenzoyloxypentane determined by mass spectrum, thereby revealing the nonsqualene nature of the center portion of the molecule.

The architecture of the fourteen carbons of the hydrocarbon terminus of the mokupalides was rigorously elucidated by spectral comparison (Table II) with 2-methyl-4-(2',6',6'-trimethylcyclohex-1'-enyl)butanal (**7**), obtained by Pd/C hydrogenation of the corresponding but-2-enal ("β-C_{14}-aldehyde").[9] The slight downfield shift of the olefinic carbons in **7** results from its aldehyde function.
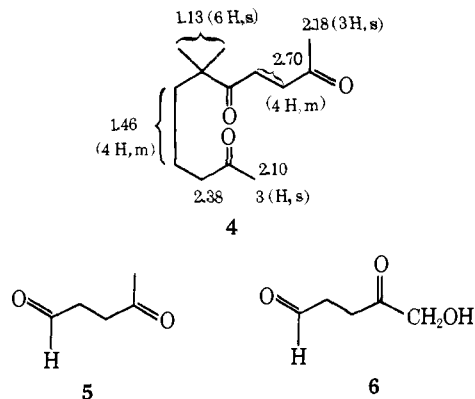
The ^{13}C NMR spectra of **1–3**, in addition to the two termini and the C-17 methylene, exhibit signals for seven allylic methylenes, three olefinic methyls, and three double bonds, trisubstituted as seen by three olefinic hydrogens, all broad triplets, in the ^{1}H NMR spectrum. ^{13}C NMR signals of the mokupalides (**1–3**) include olefinic methyls at 15.9–16.0 ppm, three olefinic peaks near 123, and three near 135 ppm. By comparison, the central olefinic methyl of squalene resonates at 16.2 ppm and the olefinic carbons (trisubstituted) at 124.9 and 134.9 ppm.[10] Furthermore, in (E)-3-methylhex-3-ene the methyl signal occurs at 15.7 ppm and in the Z isomer at 22.9.[11] The olefinic carbons of the E isomer resonate at 136.3 (C-3) and 125.2 (C-4) ppm.

Acyclic hexaprenes linked to quinoid or aromatic nuclei are prominent bimolecules, e.g., vitamins K and E and ubiquinones, and have also been isolated from a Mediterranean sponge.[22] Another sponge has given rise to a series of C_{21} furanoterpenes with the same butenolide end group.[13] The new hexaprenoids are also reminiscent of the sponge-derived pentacyclic sesterterpene scalarin.[14]

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Received July 18, 1977

p-Toluenesulfonyldiazoacetates as Photoaffinity Labeling Reagents

Sir:

Photoaffinity labeling has become an important method for the identification of target sites in biological systems, as well as for marking the amino acid residues in the vicinity of the active sites of enzymes.[1] Many new reagents and some new procedures have been invented since the method was introduced[2] in 1962. The diazo functionality has been incorporated into many of the reagents for photoaffinity labeling and is advantageous insofar as the carbenes produced on photolysis are highly reactive and insert promptly into any nearby bond, including C–H bonds.[3] Nevertheless, most of the diazo esters previously employed have suffered from two defects: instability toward heat and acid, and loss of reagent during photolysis because of Wolff rearrangement. To achieve effective labeling, the carbene produced on photolytic decomposition of diazo compounds must insert into surrounding molecules or amino acid residues; when, however, photolysis of a diazo ester leads to Wolff rearrangement, it produces a ketene which generally[4] reacts with solvent; if it does so, the product provides no new information concerning the enzyme or other biological target. The Wolff rearrangement consumes (and therefore wastes) an important fraction of the photoproduct from ordinary diazo ester and all of it from most diazo esters of thiols.[5]

New configurations for the diazo functionality are therefore needed where the compounds are thermally stable, insensitive to acid, and not subject to Wolff rearrangement. This communication reports the preparation of compounds which, to a large extent, meet these requirements. We have examined the photolysis of ethyl 2-diazo-2-*p*-toluenesulfonylacetate,[6] A, of ethyl 2-diazo-2-*p*-toluenesulfonylthioacetate, B, and of related *p*-toluenesulfonyldiazoacetates. These serve as models for proteins derivatized with the sulfonyldiazoacetyl group. Further, we have prepared and characterized two reagents that will be of use in derivatizing thiol or activated hydroxyl groups: 2-diazo-2-*p*-toluenesulfonylacetyl chloride, C, and *p*-nitrophenyl 2-diazo-2-*p*-toluenesulfonyl acetate, D. The reagents themselves are apparently indefinitely stable on storage at room temperature; the ester, A, is unaffected by 1 M acid. The *O*-ethyl ester, A, undergoes photolysis at long wavelengths in methanol with at least 95% insertion into the –OH bond of the solvent; no product of Wolff rearrangement was detected. Furthermore, the extinction coefficient of ethyl 2-diazo-2-*p*-toluenesulfonylacetate is 140 at 370 nm, whereas that for ethyl diazoacetate[7] is only 14. The greater extinction coefficient leads to rapid photolysis and may allow photolysis in reasonable times in those cases where the biological molecules to which the reagent is attached are destroyed by short wavelength ultraviolet. See Figure 1.

The principal product (90–95% yield) of the photolysis at 2537 or 3500 Å of ethyl 2-diazo-2-*p*-toluenesulfonylacetate in methanol is E (R = CH$_3$), of insertion of the carbene, produced by photolysis, into the –OH bond of methanol. The principal minor products are shown below.
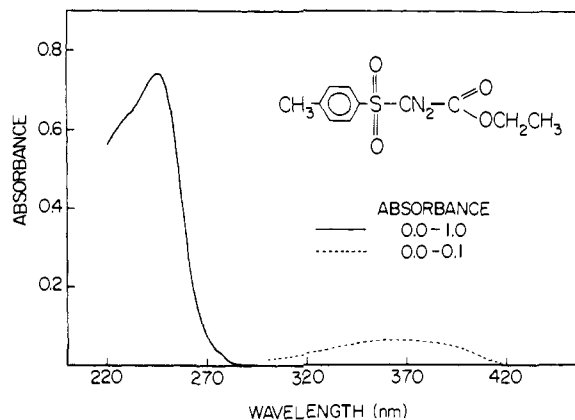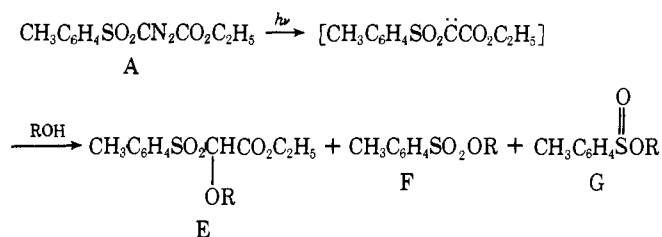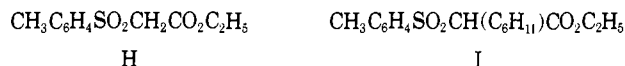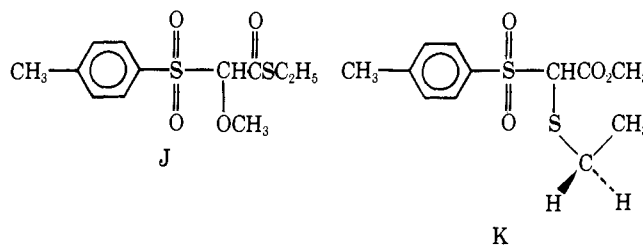

Figure 1. Ultraviolet spectrum of a 5.04×10^{-5} M solution of ethyl 2-diazo-2-*p*-toluenesulfonylacetate. Note change of absorbance scale at 300 nm.

$$CH_3C_6H_4SO_2CN_2CO_2C_2H_5 \xrightarrow{h\nu} [CH_3C_6H_4SO_2\ddot{C}CO_2C_2H_5]$$

A

$$\xrightarrow{ROH} CH_3C_6H_4SO_2CHCO_2C_2H_5 + CH_3C_6H_4SO_2OR + CH_3C_6H_4SOR$$

(E, with OR substituent) F G

Photolysis of A in ethanol leads, in addition to E, F, and G (R = C$_2$H$_5$), to the reduced compound, H. The photolysis of A in cyclohexane solution, either at 2537 or at 3500 Å, led to the formation of I (90% yield).

$$CH_3C_6H_4SO_2CH_2CO_2C_2H_5 \qquad CH_3C_6H_4SO_2CH(C_6H_{11})CO_2C_2H_5$$

H I

The photolysis of the *p*-toluenesulfonyldiazoacetates of thiols is of interest because simple diazoacetates of thiols[5] undergo complete Wolff rearrangement.[8] When the photolysis of B was carried out at 3500 Å, the products consisted of 25% J, the product of insertion into the solvent, and 68% K, the product of Wolff rearrangement. The sulfonate and sulfinate, F and G (R = CH$_3$), occur as minor by-products.

J   K

The acid chloride, C, was prepared in 46% yield by mixing 710 mg of *p*-toluenesulfonyldiazomethane,[9] 0.9 mL of phosgene, and 775 mg of Proton Sponge in 50 mL of methylene chloride in the dark at Dry Ice temperatures. The black reaction mixture was warmed to room temperature overnight, washed in ether solution with acid and water, dried, and evaporated. The resulting brown solid was chromatographed in toluene–hexane (3:1) over 35 g of Woelm grade II silica gel. The product, recrystallized from methylene chloride–hexane, melted at 107–108 °C.[10] The *p*-nitrophenyl ester, D, was prepared in 83% yield by stirring 150 mg of C with 112 mg of dried sodium *p*-nitrophenylate for 8 h at room temperature under nitrogen in 50 mL of methylene chloride. Evaporation of the filtered solution yielded yellow crystals that, after re-