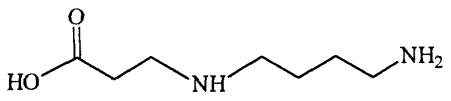
SYNTHESIS OF LIPOPHILIC 1-SUBSTITUTED DIAZEN-1-IUM-1,2-DIOLATES

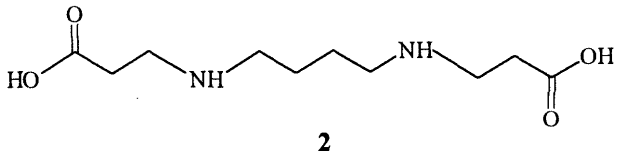
Dominick C. ROSELLE* and Daniel J. SMITH
The Department of Chemistry, The University of Akron, Akron, Ohio, 44325-3601, U.S.A.

A new class of 1-substituted diazen-1-ium-1,2-diolates (nitric oxide donors previously known as NONOates) were obtained by preparing esters of the naturally occurring polyamines putreanine (**1**) and spermic acid (**2**) with either cholesterol or hexadecanol. The syntheses of the following compounds and the corresponding NONOates are reported: cholesterylputreanine (**5**), dicholesterylspermate (**6**), hexadecanylputreanine (**7**), and dihexadecanylspermate (**8**).

KEYWORDS Nitric Oxide; Nitric Oxide Release; 1-Substituted Diazenium Diolates; NONOates; Lipophilic Polyamines



1

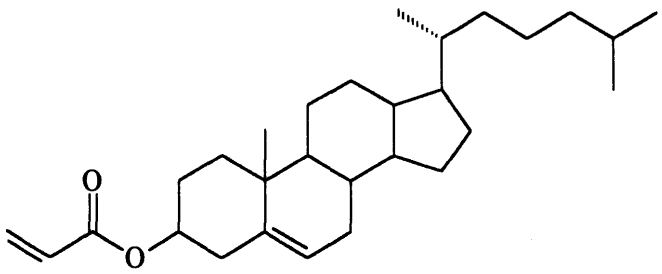


2

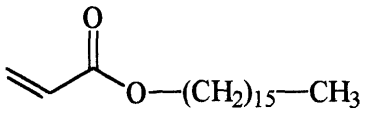
Recently 1-substituted Diazen-1-ium-1,2-diolates (previously known as NONOates), a new class of nitric oxide donating compounds, have been described by Kieffer et al. These water soluble NONOate compounds are able to undergo spontaneous degradation when placed in aqueous buffers thus releasing two equivalents of nitric oxide. The reaction is acid catalyzed and pH dependent (1).

A pharmacological need has arisen for water insoluble NONOates which are able to display a more sustained release of nitric oxide. Thus, the lipophilic and water insoluble cholesterol and hexadecanol esters of the natural polyamines putreanine and spermic acid, as well as their corresponding NONOates, were synthesized and characterized.

Cholesterylacrylate (**3**) and hexadecanylacrylate (**4**) were synthesized by heating cholesterol or hexadecanol with 1.2 equivalents of acryloylchloride in benzene at 80°C for 24 hours (2). Cholesterylacrylate was recrystallized using ether/ethanol (2) and hexadecanylacrylate was purified by extracting with 10% $NaHCO_3$ to remove and neutralize unconsumed acryloylchloride, followed by removal of the benzene by rotoevaporation and vacuum oven drying at room temperatue. The products were obtained in 75% yields, and purities were confirmed by ^1H NMR.



3

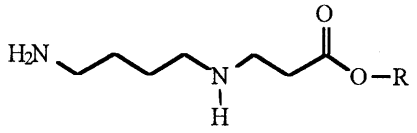


4

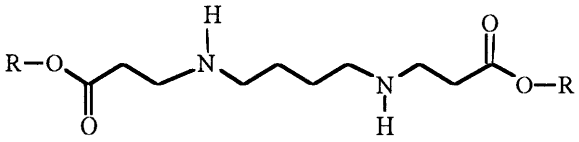
Esters of putreanine (**1**) were prepared by adding dropwise compounds **3** or **4**, dissolved in THF, to a five molar excess of 1,4-diaminobutane in THF to form compounds **5** and **6** (3). Compounds **5** and **6** were first obtained in 50% yields by extraction into hexane, and characterized by ^1H NMR analysis. Diesters of spermic acid (**2**) were prepared by adding two equivalents of **3** or **4** to 1,4-diaminobutane as

* To whom correspondence should be addressed.

described above. Compounds **7** and **8** were obtained in 90% yields and examined by ^{1}H NMR analysis. The synthesized compounds are basically ester derivatives of the naturally occurring polyamines putreanine and spermic acid; thus, they were easily characterized by comparing their ^{1}H NMR spectra to that of putreanine **(1)** and spermic acid **(2)** standards. All synthesis and purification steps of the polyamine esters were conducted at or below room temperature since compound degradation and instability were observed upon exposure to elevated temperatures.



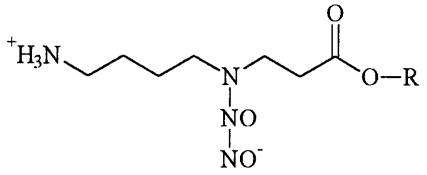
5 R = cholesterol
6 R = hexadecanol



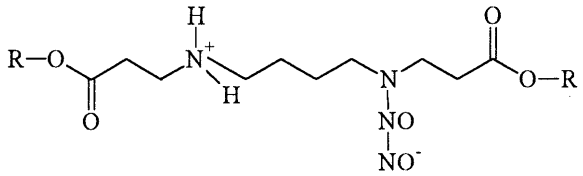
7 R = cholesterol
8 R = hexadecanol

Compounds **5**, **6**, **7**, or **8** were converted to the corresponding 1-substituted diazen-1-ium-1,2-diolate **(9,10, 11, 12)** (previously known as NONOates) using THF as the solvent. The reactions were done in a high pressure glass bottle charged with 5 atmospheres of nitric oxide which were allowed to react for 48 hours as previously described (4). The products were isolated by removing the THF solvent by roto-evaporation at room temperature, all final traces of solvent were removed by evaporation in a room temperature vacuum oven.

The compounds **9, 10, 11,** and **12** were characterized by ^{1}H NMR which showed total conversion to product thus enabling yields of 90% and better. In all cases, the ^{1}H NMR spectrum for the NONOates of CP, HP, CS, and HS consistently illustrated the generation of four new and distinct resonances (2.8-4.4 ppm) farther upfield after modification with nitric oxide (Table 4). For CP, HP, and HS, the disappearance of two resonances from the putreanine region of the spectrum (1.3-2.6 ppm) was observed but for CS, only the disappearance of the resonance at 2.62 ppm could be observed, any other changes could not be resolved from the spectrum of cholesterol in the region of 0-2 ppm. The NMR analysis also showed that no reactions occurred at the vinyl group of cholesterol nor at the ester linkages; also, only one product was ever obtained, with no unreacted starting materials or side products present. Extinction coefficients (calculated from absorbances at 240 nm), using THF as the solvent, were determined and in close agreement with published values for water soluble NONOates (1).



9 R = cholesterol
10 R = hexadecanol



11 R = cholesterol
12 R = hexadecanol

A Monitor Labs model 8440 Nitric Oxide Analyzer, interfaced with an HP 3396 A Chromatography Integrator, was used to measure nitric oxide release from the experimental compounds. The analyzer was connected to a release chamber consisting of an impinger bottle (Ace Glass) which had one way Teflon stop cock valves attached in order to prevent the escape of any generated nitric oxide. The amount of liberated NO was determined by the daily or timely flushing of helium through the solution and into the analyzer. Typically, measurements were taken every 2-4 hours during the first 18 hours and then every 12 hours after that. Eventually, readings needed to be taken only every 24 hours as the end of the release profile was approached. Helium gas was flushed through the system at 12 psig which was regulated by a Teflon flow meter set at 150 ml/min.

The release of nitric oxide was examined using phosphate buffered saline (PBS) at pH 7.4 and with the addition of Tween 20 or the therapeutic lung surfactant Survanta® (Abbott Laboratories, Inc.), both of which facilitated emulsification of the compounds in aqueous buffers (Tables 1 and 2). Nitric

oxide release was found to be first ordered and half-lives were several days longer than those of the first generation water soluble NONOates (Table 3).

Table 1. Influence of Survanta on Nitric Oxide Release in PBS pH 7.4.

Compound	half-life PBS	half-life 1% Survanta	half-life 5% & 10% Survanta
CPNO	60 hours	62 hours	39 hours
DiHSNO	7 days	9 days	not determined

Table 2. Influence of Tween 20 on Nitric Oxide Release in PBS pH 7.4.

Compound	half-life PBS	half-life 5% Tween 20	half-life 10% Tween 20
CPNO	60 hours	104 hours	72 hours
HPNO	81 hours	89 hours	108 hours

Table 3. Half-lives for Nitric Oxide release of water soluble NONOates in PBS pH 7.4. (Adapted from Maragos, et al.) (Common Names: DETA-NO: Diethylenetriamine-NONOate sodium salt; SPER -NO: Spermine-NONOate; PAPA-NO: Aminopropyl-propylamine-NONOate; DEA-NO: Diethylamine-NONOate; MAHMA-NO: Methylamino-hexyl-methylamine-NONOate)

Compound	half-life
DETA-NO	57 hours
SPER-NO	230 minutes
PAPA-NO	77 minutes
DEA-NO	16 minutes
MAHMA-NO	3 minutes

Table 4. Spectroscopic data of **5-12**

5: ^{1}H NMR (600 MHz, THF-d_8): (Excludes chemical shifts for aliphatic cholesterol region) δ=2.30 (2H), 2.38 (XH), 2.51 (2H), 2.63 (2H), 2.80 (2H), 4.59 (1H), 5.36 (1H).

6: ^{1}H NMR (300 MHz, $CDCl_3$): δ=0.75 (3H), 1.13 (28H), 1.38 (4H), 1.51 (2H), 2.40 (2H), 2.51 (2H), 2.58 (2H), 2.76 (2H), 3.97 (2H).

7: ^{1}H NMR (300 MHz, $CDCl_3$): (Excludes chemical shifts for aliphatic cholesterol region) δ=2.29 (2H), 2.48 (2H), 2.62 (2H), 2.86 (2H), 4.60 (1H), 5.36 (1H).

8: ^{1}H NMR (600 MHz, THF-d_8): δ=0.89 (3H) , 1.29 (26H), 1.46 (2H), 1.61 (2H), 2.40 (2H), 2.57 (2H), 2.80 (2H), 4.02 (2H).

9: ^{1}H NMR (600 MHz, THF-d_8): (Excludes chemical shifts for aliphatic cholesterol region) δ=2.30 (2H), 2.40 (2H), 2.88 (2H), 3.41 (2H), 3.73 (2H), 4.22 (2H), 4.35 (2H), 4.61 (1H), 5.37 (1H).

10: ^{1}H NMR (300 MHz, $CDCl_3$): δ=0.89 (3H), 1.19 (28H), 1.61 (2H), 2.55 (2H), 2.88 (2H), 3.65 (2H), 3.79 (2H), 4.02 (2H), 4.24 (2H), 4.35 (2H).

11: ^{1}H NMR (300 MHz, $CDCl_3$): (Excludes chemical shifts for aliphatic cholesterol region) δ=2.29 (4H), 2.52 (4H), 2.82 (4H), 3.63 (2H), 3.75 (2H), 4.21 (2H), 4.34 (2H) , 4.62 (1H), 5.36 (1H).

12: ^{1}H NMR (600 MHz, THF-d_8): δ=0.89 (3H), 1.29 (26H), 1.62 (2H), 2.47 (2H), 2.81 (2H), 3.71 (1H), 4.03 (2H), 4.15 (1H), 4.23 (1H), 4.34 (1H).

REFERNCES

1) Hrabie J.A., Klose J.R., Wink D.A., Keefer L.K., *J. Org. Chem.*, **58**, 1472-1476 (1993).
2) De Visser A.C., De Groot D.E., Feyen J., Bantjes A., *J. Polym. Sci.*, A-1, **8**, 1893-1894 (1971).
3) Asahara T., *Die Makromolekulare Chemie*, **136**, 211-219 136 (1970).
4) Maragos C.M., Morley D., Wink D.A., Dunams T.M., Saavedra J.E., Hoffman A., Bove A.A., Isacc L., Hrabie J.A., Keefer L.K., *J. Med. Chem.*, **34**, 3242-3247 (1991).

(Received December 27, 1996; accepted March 18, 1997)