Communications to the Editor

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SYNTHESIS AND BIOLOGICAL ACTIVITIES OF BIOISOSTERIC O-CARBA-ANALOGUES OF PLATELET ACTIVATING FACTOR (PAF)

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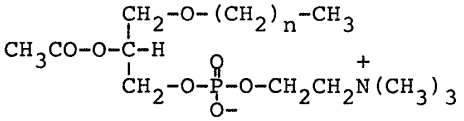
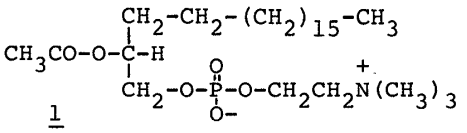
Four types of O-carba-analogues of C_{16}-PAF were synthesized: dl-2-acetoxyeicosylphosphoryl choline (1), dl-3-hexadecyloxy-2-(2-oxopropyl)propylphosphoryl choline (2), dl- and (S)-(-)-3-acetoxy-4-hexadecyloxybutylphosphonyl choline (3), and dl-2-acetoxy-3-hexadecyloxypropyl (3-trimethylammonio)propylphosphonate (4). Both in hypotension and in platelet aggregation, (S)-(-)-3 was one third as potent as synthetic C_{16}-PAF, whereas 1, 2 and 4 were 100-3,000 times less potent.

KEYWORDS —— platelet activating factor; 1-O-carba-PAF; 2-O-carba-PAF; 3-O-carba-PAF; hypotension; platelet aggregation

Since the structure of the first physiologically active phospholipid, platelet activating factor (PAF)[1] or antihypertensive polar renomedullary lipid (APRL),[2] was elucidated, a number of its derivatives have been synthesized in search of compounds with more selective biological activities. With the concept of bioisosterism[3] in mind, we synthesized O-carba-derivatives of C_{16}-PAF,[4,5] 1 - 4. We report here the synthesis of these isosteres, and their hypotensive and platelet aggregating activities in comparison with synthetic C_{16}- and C_{18}-PAF.[6]





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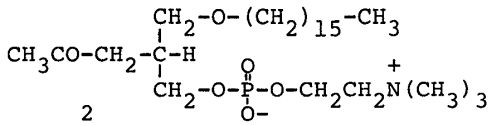

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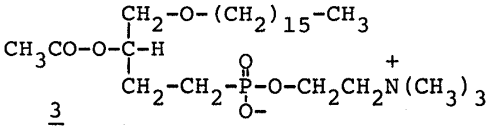

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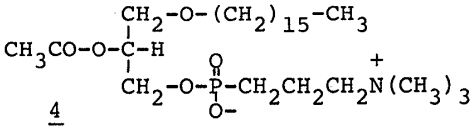

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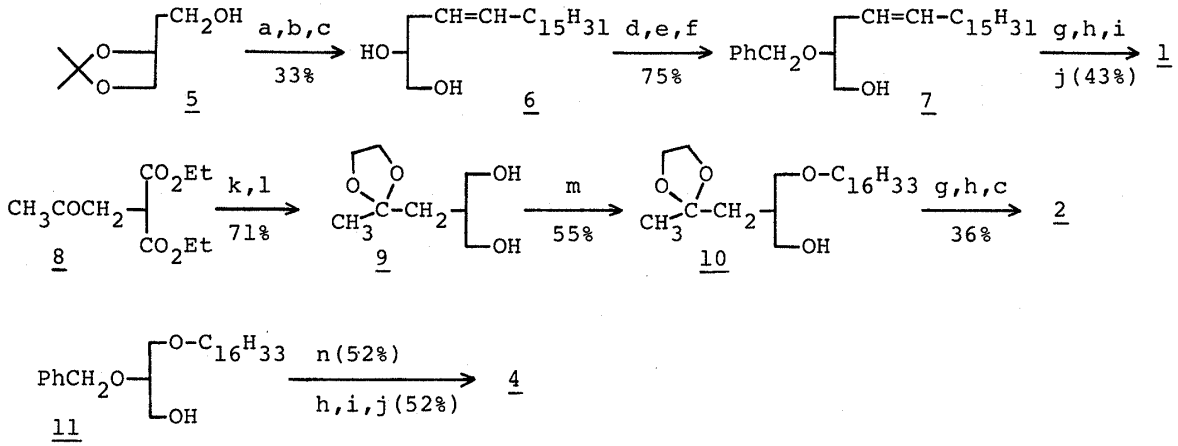
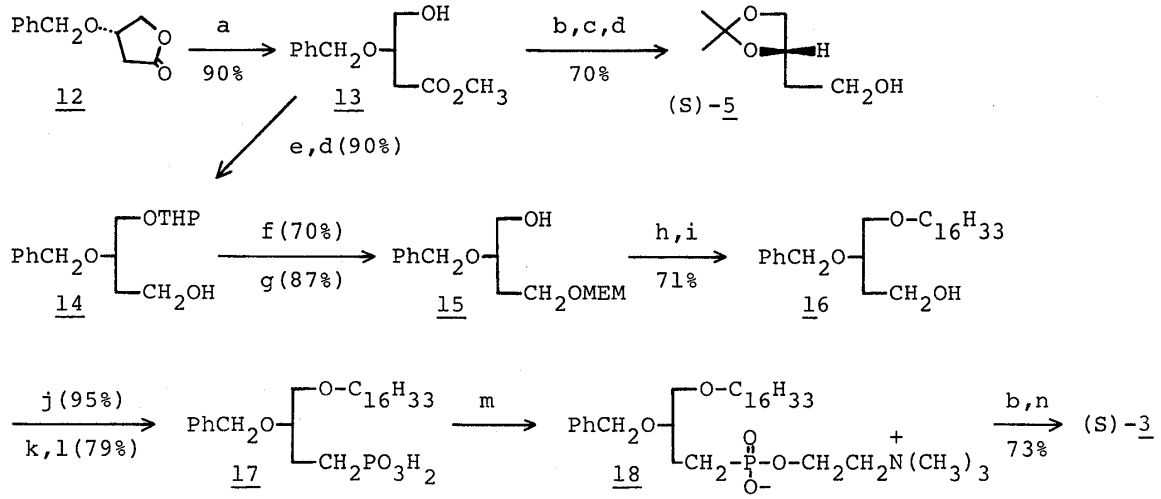
The synthesis of racemic 1, 2 and 4 is outlined in Chart 1. The diol 6 (mp 54.5-56°C)[9] was prepared from pure 5.[7] According to the known procedures,[6,8] 6 was converted into dl-1[9] (amorphous powder, mp 230-235°C). Acetalization and reduction of known 8[10] afforded the diol 9[9] [bp 120°C(1 mmHg)], which was monoalkylated to 10[9] [bp 180-185°C (4 mmHg)]. Introduction of the choline phosphate moiety into 10 in the usual manner, followed by deacetalization, yielded dl-2[9] (amorphous powder, mp 63-66°C). Condensation of dl-11 with 3-bromopropylphosphonodichloridate followed by the usual procedures as described in the synthesis of natural PAF[6,8] gave dl-4[9] (amorphous powder, mp 39-42°C).



a) PCC, NaOAc; b) n-BuLi, n-$C_{16}H_{33}PPh_3Br$, THF, -78°C; c) aq. AcOH; d) Ph_3CCl, C_5H_5N; e) NaH, $PhCH_2Cl$; f) p-TsOH, MeOH; g) $BrCH_2CH_2OPOCl_2$, Et_3N, H_2O; h) Me_3N, Ag_2CO_3; i) H_2, Pd-C; j) Ac_2O, Et_3N; k) $HOCH_2CH_2OH$, BF_3-Et_2O, CH_2Cl_2; l) $LiAlH_4$, THF, r.t.; m) NaH, 1 eq n-$C_{16}H_{33}Br$, DMF, r.t., 3 h; n) Et_3N, $BrCH_2CH_2CH_2POCl_2$, Et_2O, r.t., 20 h, H_2O

Chart 1

Chart 2 illustrates the synthesis of 3-O-carba-PAF, (S)-(-)-3, starting from (S)-12,[11] $[\alpha]_D^{25}$ -29.7° (c=1.00, $CHCl_3$). Acidic treatment of 12 in methanol gave the ester 13, $[\alpha]_D^{25}$ -2.42° (c=1.20, $CHCl_3$).[9] To confirm the structure and the optical purity, 13 was converted into the well known chiral synthon, (S)-(-)-5, $[\alpha]_D^{25}$ -2.50° (c=9.8, MeOH) [lit.[7] $[\alpha]_D$ -2.23° (c=9.8, MeOH)]. Since the earlier preparation of 5 has been plagued with the formation of an isomeric six-membered acetal,[7] the present method provides an alternative route for 5 from malic acid. As attempts to alkylate 13 with n-hexadecyl bromide under various basic conditions failed because of the elimination of the benzyloxy group, 13 was converted into the methoxyethoxymethyl ether 15,[9] $[\alpha]_D^{25}$ -1.01° (c=1.09, $CHCl_3$). Alkylation of 15 followed by removal of the protecting group afforded 16,[9] $[\alpha]_D^{25}$ -24.52° (c=1.04, $CHCl_3$). Bromination of 16 and the subsequent Arbuzov reaction of the resulting bromide with tris(trimethylsilyl) phosphite[12] yielded the phosphonic acid 17.[9] Condensation of 17 with choline tosylate[13] gave 18,[9] $[\alpha]_D^{25}$ -1.15° (c=1.04, $CHCl_3$), which was debenzylated and acetylated to afford (S)-(-)-3[9] [resinous oil; FAB-MS, QM^+ 522 (M+H), $[\alpha]_D^{25}$ -1.18° (c=0.93, $CHCl_3$-MeOH, 1:1)]. Racemic 3 (resinous oil) also was synthesized similarly.



a) CSA, MeOH, reflux, 15 h; b) H_2, Pd-C, MeOH; c) $Me_2C(OMe)_2$, p-TsOH; d) $LiAlH_4$, THF; e) DHP, PPTS; f) NaH, MEMCl, DMF, 40°C, 3 h; g) aq. AcOH; h) $n\text{-}C_{16}H_{33}Br$, KOH, C_6H_6, reflux, 18 h; i) conc. aq. HCl-MeOH, 50°C, 5 h; j) CBr_4, Ph_3P, CH_2Cl_2, r.t., 1 h; k) $P(OSiMe_3)_3$, 150°C, 22 h; l) EtOH; m) choline tosylate, Cl_3CCN, C_5H_5N, 50°C, 11 h; n) Ac_2O, Et_3N

Chart 2

The hypotensive effects of 1 - 4 were tested in Wistar-Imamichi rats anesthetized by intraperitoneal injection of pentobarbital. The compounds dissolved in saline containing o.25% bovine serum albumin were intravenously administered. The hypotensive response was recorded with a pressure transducer which was connected to a cannula placed in the femoral artery. The response was evaluated on the basis of an index, the maximum reduction of the mean blood pressure (mmHg) x time required for a 50% recovery (min). The dose response curve was constructed, and the relative potency was calculated from the dose which caused a response equivalent to that induced by 0.1 μg/kg of C_{16}-PAF (synthesized from D-mannitol according to Godfroid's method for C_{18}-PAF[6]).

The platelet aggregating activities were measured by Born's method,[14] using rabbit blood collected by directly puncturing the heart. Platelet rich plasma (PRP) and platelet poor plasma (PPP) were prepared by centrifuging the blood, and the platelet concentration of the test plasma was adjusted to 600,000/μl by mixing the PRP and PPP. The EC_{50} values were calculated from the concentration-aggregation curves. The biological data are summarized in Table I.

Table I. Biological Activities of Carba-Analogues of C_{16}-PAF

Compound	C_{16}-PAF	C_{18}-PAF	dl-1	dl-2	(S)-3	dl-3	dl-4
Hypotensive Activity (%)	100	33	0.03	0.1-0.3	33	10-33	1.0
Platelet Aggregating Activity (EC_{50} μM)	0.009	0.027	1.20	0.16	0.027	0.044	0.08

Replacement of the sn-1, sn-2 or choline oxygen atom in the molecule of C_{16}-PAF with a methylene group markedly reduced both activities, whereas replacement of the sn-3 oxygen atom decreased the activities only slightly, leaving a potency comparable to that of C_{18}-PAF. This fact suggests that the sn-3 oxygen atom plays a less important role than other ether or ester oxygen atoms.

Since PAF is inactivated by the enzymatic hydrolysis of the acetoxy group, the 2-O-carba-derivative 2 was expected to have a long-acting hypotensive effect. This, however, was not the case: 2 (100 μg/kg) produced a short-lasting hypotension similar to that induced by C_{16}-PAF (0.1 μg/kg).

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