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AN EFFICIENT SYNTHESIS OF PLATELET-ACTIVATING FACTOR (PAF) VIA 1-O-ALKYL-2-O-(3-ISOXAZOLYL)-SN-GLYCERO-3-PHOSPHOCHOLINE, A NEW PAF AGONIST. UTILIZATION OF THE 3-ISOXAZOLYLOXY GROUP AS A PROTECTED HYDROXYL.

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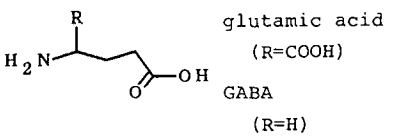
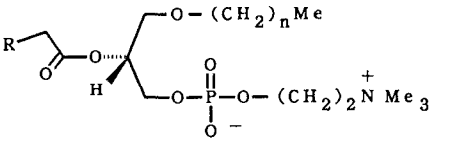
Summary: Potent PAF agonists (R)-**3a,b** were synthesized and converted into PAF. Key steps include Mitsunobu reaction of a chiral secondary alcohol with 3-hydroxyisoxazole as the acidic component, and hydrogenolytic ring cleavage of the resulting 3-isoxazolyloxy group into the inverted alcohol.

Platelet-activating factor (PAF) (**1**) is an alkyl ether phospholipid mediator that plays important roles in various biological and pathological processes[1)]. According to the structure-activity relationships of PAF analogues so far reported, a small acyloxy or carbamoyloxy group is necessary at the sn-2 position to exert potent agonistic activities[2)].

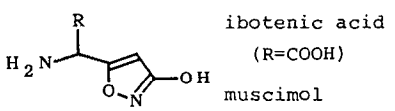
Two natural 3-hydroxyisoxazoles, ibotenic acid and muscimol, have been reported to be potent agonists of neurotransmitters glutamic acid and GABA, respectively[3,4)]. This bioisosterism between 3-hydroxyisoxazole and propionic acid structures prompted us to synthesize 2-(3-isoxazolyloxy)- PAF derivatives (R)-**3a,b** corresponding to propionyl PAF[5)] (**2**), which is nearly equipotent as acetyl PAF (**1**), expecting agonistic activities.



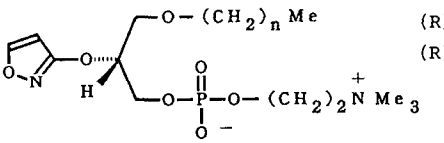
glutamic acid (R=COOH)
GABA (R=H)



1 (R=H)
2 (R=Me)
(n=15, 17)



ibotenic acid (R=COOH)
muscimol (R=H)



(R)-**3a** (n=15)
(R)-**3b** (n=17)

Our strategy was to introduce the 3-isoxazolyloxy moiety at the sn-2 position of glycerol by Mitsunobu reaction[6)] using 3-hydroxyisoxazole as the acidic component, and then cleave the ring to generate the inverted chiral alcohol, thus enabling (R)-**3a,b** to be converted into **1**.

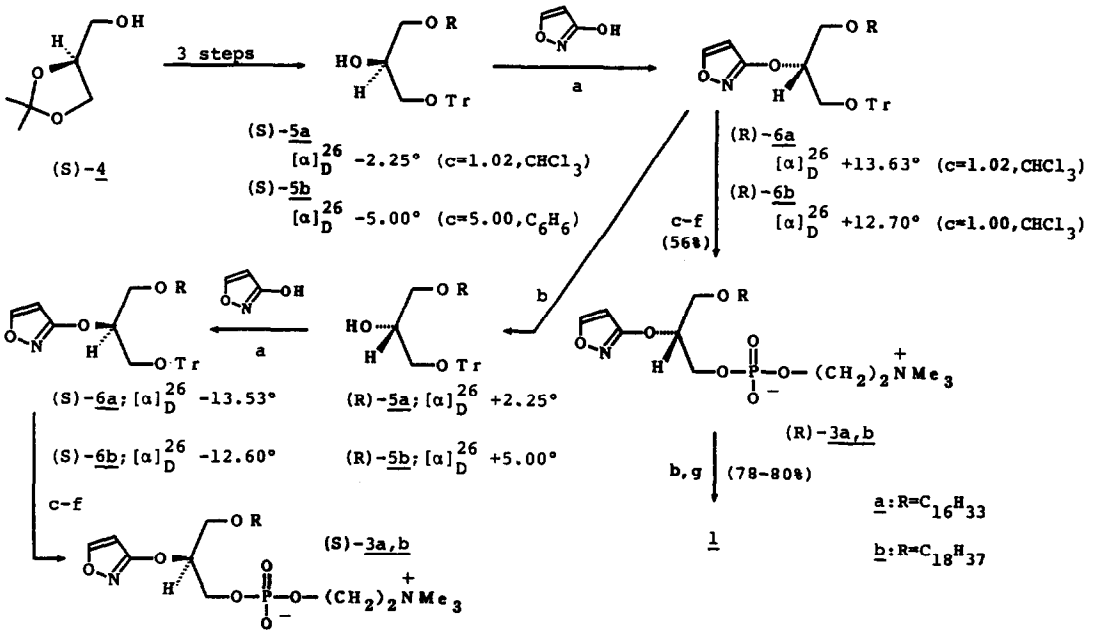
The starting material, glycerol acetonide (S)-**4** {$[\alpha]_D^{25}$ +14.6° (neat); lit.[7], $[\alpha]_D$ +14.5° (neat)}, was prepared[7] from D-mannitol. This chiral acetonide has often been criticized for racemization[8,9]. We found, however, that (S)-**4** showed the above specific rotation value after several months on storage, if the substance was carefully worked up at pH 7.2 and treated with aqueous sodium hydrogen carbonate solution before distillation. (S)-**4** was converted into (S)-**5a,b** in three steps according to Godfroid *et al.*'s method[10]. Treatment of (S)-**5a** (10 mmol) with 3-hydroxyisoxazole[11] (12 mmol), triphenylphosphine (20 mmol), and dimethyl azodicarboxylate (Tokyo Kasei, 20 mmol) in tetrahydrofuran (THF, 45 ml) at room temperature afforded the 3-*O*-alkylated isoxazole (R)-**6a** in a 87% yield after chromatography. Similarly, (R)-**6b** was obtained in a 91% yield[12]. Generally, alkylation of 3-hydroxyisoxazoles under various conditions gives a mixture of *O*- and *N*-alkylated products in a 2:1 - 1:4 ratio[3,13,15]. This first example of Mitsunobu reaction[6] using 3-hydroxyisoxazole as the acidic component provides a solution to this problem[14].

Reductive cleavage of the oxygen-nitrogen bond of the isoxazole ring has been well known. To our knowledge, however, only one example of such cleavage of 3-alkoxyisoxazole has been reported: in their synthesis of tetracycline[15], Stork and Hagedorn III catalytically hydrogenated a 3-benzyloxyisoxazole derivative in THF-MeOH, to generate a β-ketocarboxamide. In this reaction the benzyl group was probably reduced to toluene, because the amide was obtained without hydrolytic treatment. On the other hand, catalytic hydrogenation of (R)-**6a,b** (10% Pd-C, 4 atm, room temperature, 48 hr) in THF-EtOH (1:9) gave the alcohols (R)-**5a,b** in a 93-94% yield. Presumably the intermediary imino-ester of (R)-**5a,b** was decomposed by ambient EtOH. This is the first experiment to demonstrate that a 3-isoxazolyl alkyl ether can be utilized as a protected alcohol.

Repetition of the above Mitsunobu reaction with (R)-**5a,b** afforded (S)-**6a,b**. Judging from the specific rotation values of enantiomeric pairs of **5a,b** and **6a,b** (indicated in Scheme 1), the Mitsunobu reaction proceeded each time with at least 99% inversion.

Removal of the trityl group in (R)-**6a,b** and introduction of the phosphocholine side chain into the resulting alcohol were carried out according to the standard procedure reported for the corresponding (R)-2-*O*-benzyl glycerol derivatives[8,10], yielding (R)-**3a** {$[\alpha]_D^{26}$ -5.00° (c=1.00, $CHCl_3$)} and (R)-**3b** {$[\alpha]_D^{26}$ -4.70° (c=1.00, $CHCl_3$)}. Similarly, (S)-**3a** {$[\alpha]_D^{26}$ +5.00° (c=1.00, $CHCl_3$)} and (S)-**3b** {$[\alpha]_D^{26}$ +4.70° (c=1.00, $CHCl_3$)} were prepared from (S)-**6a,b**.

The isoxazole ring of (R)-**3a,b** was cleaved as described above, and the



a) Ph_3P, $(NCO_2Me)_2$; b) H_2, 10% Pd-C, EtOH; c) p-TsOH;

d) $Br(CH_2)_2OPOCl_2$, NEt_3; e) H_2O; f) NMe_3, Ag_2CO_3; g) Ac_2O, NEt_3 (Scheme 1)

resulting lyso-C_{16}-PAF {$[\alpha]_D^{25}$ -6.25° (c=1.04, $CHCl_3$:MeOH=1:1); lit.[8], $[\alpha]_D^{20}$ -6.03° (c=1.04, $CHCl_3$:MeOH=1:1)} and lyso-C_{18}-PAF {$[\alpha]_D^{26}$ -6.72° (c=5.00, $CHCl_3$:MeOH=1:1); lit.[16], $[\alpha]_D^{25}$ -6.7° (c=5, $CHCl_3$:MeOH=1:1)} were acetylated to afford C_{16}-PAF {$[\alpha]_D^{26}$ -3.70° (c=1.00, $CHCl_3$); lit.[8], $[\alpha]_D^{21}$ -3.66° (c=0.71, $CHCl_3$)} and C_{18}-PAF {$[\alpha]_D^{26}$ -4.10° (c=1.00, $CHCl_3$); lit.[8], $[\alpha]_D^{25}$ -4.00° (c=0.71, $CHCl_3$)}, respectively. Thus, the present method provides an efficient route from the chiral building block (S)-**4** to **1** (three steps shorter than the benzyl method[10]).

PAF agonistic activities of **3a,b** were measured as described earlier[17]. As expected (R)-**3a,b** exhibited potent activities, both in rabbit platelet aggregation [EC_{50}: C_{16}-PAF, 2.5 x 10^{-8} M; (R)-**3a**, 3.5 x 10^{-8} M; (R)-**3b**, 3.3 x 10^{-8} M] and rat hypotension [relative potency: C_{16}-PAF, 1.00; (R)-**3a**, 0.88; (R)-**3b**, 0.33], whereas (S)-**3a,b** were much less active [(S)-**3a**: EC_{50}, 1.1 x 10^{-6} M; relative potency, 0.003-0.01]. These results suggest that the 3-isoxazolyl group is recognized by the PAF receptor(s) as an acetyl or propionyl group. Synthesis and biological activities of antagonistic 2-O-(3-isoxazolyl)glycerol derivatives will be published elsewhere.

[References and Notes]

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