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Synthesis of Acyl Dihydroxyacetone Phosphates and Related Derivatives

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Abstract □ The chemical syntheses of 1-*O*-palmitoyl- and 1-*O*-acetyl-2,2-dimethoxypropane-3-phosphate cyclohexylammonium salt and 1-*O*-palmitoyl-2,2-dimethoxypropane-3-*O*-phosphorylethanolamine are reported. The acyl dihydroxyacetone phosphates can serve as intermediates in the pathway for the biosynthesis of phosphatidic acid, phosphatidylcholine, phosphatidylethanolamine, and alkyl glycerolipids.

Keyphrases □ Acyl dihydroxyacetone phosphates and related derivatives—synthesis for use as biosynthesis intermediates □ Dihydroxyacetone phosphates (acyl) and related derivatives—synthesis for use as biosynthesis intermediates

Studies directed toward the chemical synthesis of *O*-alkyl dihydroxyacetone and its derivatives were reported by Piantadosi *et al.* (1, 2). In a series of papers, Snyder *et al.* (3–6) and Wykle and Snyder (7, 8) reported studies on a sequence of reactions involved in the biosynthetic pathway for *O*-alkyl glycerolipids in which dihydroxyacetone phosphate was an obligatory precursor. Other investigators (9, 10) also demonstrated the presence of the *O*-alkyl ether-synthesizing enzymes in other systems. In 1970, Snyder *et al.* (4) isolated and described a microsomal enzymic system from Ehrlich ascites cells which, in the cell-free system, is capable of synthesizing *O*-alk-1-enyl glycerolipids (plasmalogens).

DISCUSSION

In a continuation of this research, we now report on synthetic studies involving the acyl analog, which is an important intermediate in the biosynthetic scheme. Acyl dihydroxyacetone phosphate was characterized by Hajra and Agranoff (11) and found to occur in guinea pig liver. It is formed (12) enzymatically by acylation of dihydroxyacetone phosphate in the presence of acyl CoA, ATP, and Mg^{+2} as cofactors. This keto intermediate can then be reduced in mitochondria by NADPH (13) and further acylated to form phosphatidic acid. Agranoff and Hajra (14) also reported on the participation of the acyl dihydroxyacetone phosphate pathway in mouse liver and Ehrlich ascites tumor cells, and they suggested that this pathway plays an important role in these cells. Furthermore, Hajra (9) and Wykle *et al.* (15) found that acyl dihydroxyacetone serves as a precursor of *O*-alkyl glycerolipids.

In this paper, we describe the complete chemical synthesis of acyl dihydroxyacetone phosphates which is based on an earlier procedure (2) used for the synthesis of 1-*O*-alkyl dihydroxyacetone derivatives. The cyclohexylammonium salts of 1-*O*-palmitoyl-2,2-dimethoxypropane-3-phosphate (VIII, Scheme I), 1-*O*-acetyl-2,2-dimethoxypropane-3-phosphate (XII, Scheme II), and 1-*O*-palmitoyl-2,2-dimethoxypropane-3-phosphorylethanolamine (XVI, Scheme III) were prepared. In the synthesis of palmitoyl derivatives (VIII and XVI), 3-*O*-benzylglycerol (Compound I) was used as the starting material. Compound I was acylated with palmitoyl chloride in the presence of pyridine at −20°, resulting in a crude mixture of II which contained over 70% of the monopalmitoyl derivative as determined by TLC. The Compound II mixture was then subjected to the Pfitzner–Moffatt (16) oxidation to III in a solution of dicyclohexylcarbodiimide–dimethyl sulfoxide, containing a proton source such as pyridinium phosphate or pyridinium trifluoroacetate, in a manner similar to that described by Hartman (17) for the synthesis of 3-haloacetol phosphates. In this oxidation reaction, dimethyl sulfoxide is converted into a labile intermediate which facilitates the attack at the sulfur atom by the β-hydroxy group of benzylglycerol. Trifluoroacetic acid was used to initiate the reaction, while dicyclohexylcarbodiimide was used as a polarizing agent (2).

The keto compound, III, was ketalized to IV, which was debenzylated with palladium black to V. Compound V was phosphorylated with diphenyl chlorophosphate to VI and then treated with platinum oxide to remove the phenyl groups resulting in VII. Compound VII was subsequently treated with cyclohexylamine and isolated as the salt, VIII.

Compound V was also phosphorylated with phenyl phosphorodichloridate (Scheme III) to XIII, which was then reacted with carbobenzoxyethanolamine, resulting in XV. The protecting groups were removed by hydrogenation with platinum oxide and palladium black at room temperature to XVI. The acetyl derivative (IX),

prepared by the procedure of Ballou and Fischer (18), was also phosphorylated with diphenyl chlorophosphate in an analogous manner as for the preparation of VIII and isolated as the cyclohexylammonium salt, XII.

EXPERIMENTAL[1]

3-*O*-Benzylglycerol (I)—3-*O*-Benzylglycerol was prepared according to the procedure of Sowden and Fischer (19) and Howe and Malkin (20).

1-*O*-Palmitoyl-3-*O*-benzyl-2-propanone (III)—Compound I was esterified with palmitoyl chloride in the usual manner. A mixture of the mono- and dipalmitoyl derivatives was obtained. TLC, using a solvent system of *n*-hexane–diethyl ether (50:50 v/v), showed about 70% conversion into the monopalmitoyl derivative.

A mixture of 59 g. of the crude palmitoyl esters, 15 ml. of dimethyl sulfoxide, 68 g. of dicyclohexylcarbodiimide, and 3 ml. of pyridine in 500 ml. of anhydrous ether was cooled to 4° in an ice bath. The mixture was then removed from the ice bath, and 3 ml. of trifluoroacetic acid was added to initiate the oxidation reaction. After 5 hr. of stirring at room temperature, 20 g. of oxalic acid in 40 ml. of methanol was added to decompose the excess dicyclohexylcarbodiimide. Thirty minutes later, the dicyclohexylurea was removed by suction filtration and washed with ether. The combined filtrate was extracted three times with 200-ml. portions of saturated sodium bicarbonate solution and two 200-ml. portions of water and was finally dried over anhydrous sodium sulfate. The ether was removed under reduced pressure, and the residue was dissolved in 300 ml. of ether and left overnight to remove any residual dicyclohexylurea. The ether was removed, and the residue was dissolved in 250 ml. of *n*-hexane and kept overnight at 5°. The resulting crystals were filtered, washed with ice-cold *n*-hexane, and dried under vacuum over phosphorus pentoxide. The crystals (26 g.) were recrystallized from 120 ml. of *n*-hexane at 5° to yield 18 g. of product (42%, based on the reacted 3-*O*-benzylglycerol), m.p. 50–51°. TLC, using a solvent system of *n*-hexane–diethyl ether (80:20 v/v), showed one spot having an R_f value of 0.60.

Anal.—Calc. for $C_{26}H_{42}O_4$: C, 74.59; H, 10.11. Found: C, 74.89; H, 10.12.

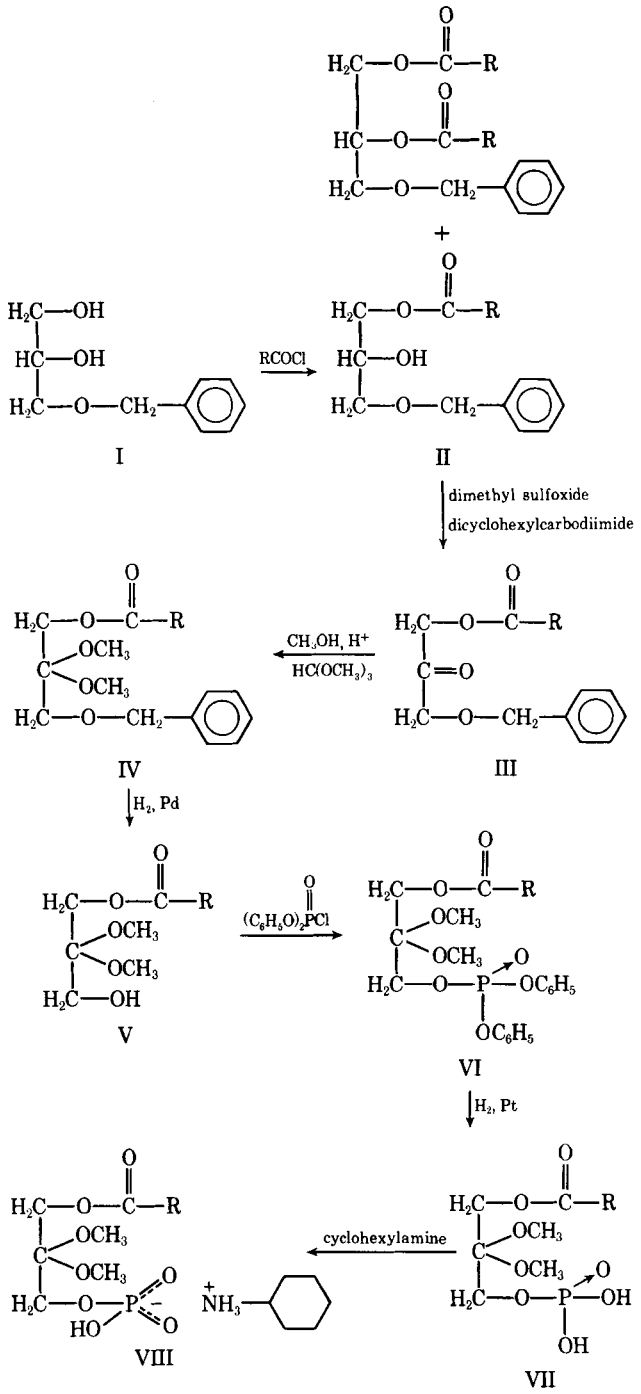
1-*O*-Palmitoyl-2,2-dimethoxy-3-*O*-benzylpropane (IV)—Compound III was ketalized in the presence of absolute methanol, trimethyl orthoformate, and a catalytic amount of concentrated sulfuric acid.

Anal.—Calc. for $C_{28}H_{48}O_5$: C, 72.37; H, 10.41. Found: C, 72.42; H, 10.28.

1-*O*-Palmitoyl-2,2-dimethoxy-3-hydroxypropane (V)—A solution of 9.0 g. of Compound IV in 150 ml. of *n*-hexane was debenzylated by shaking with 2 g. of palladium black at 20 p.s.i. of hydrogen at room temperature for 5 hr. The crude product (7.2 g.) was purified by silica gel column chromatography. The material was dissolved in 10 ml. of warm *n*-hexane and applied on 210 g. of silica gel in a column of 3.7-cm. diameter. Five fractions were eluted and collected using the following solvent systems: Fraction I, 300 ml. *n*-hexane; Fraction II, 300 ml. *n*-hexane–diethyl ether (95:5 v/v); Fraction III, 300 ml. *n*-hexane–diethyl ether (90:10 v/v); Fraction IV, 300 ml. *n*-hexane–diethyl ether (85:15 v/v); and Fraction V, 300 ml. *n*-hexane–diethyl ether (80:20 v/v). Fractions I and II contained nonpolar contaminates, Fraction III contained some of the unreacted Compound IV, Fraction IV contained traces of Compound III, and Fraction V contained 6.0 g. of Compound V (81% yield); m.p. 45–46°.

Anal.—Calc. for $C_{21}H_{42}O_5$: C, 67.34; H, 11.30. Found: C, 67.43; H, 11.29.

Cyclohexylammonium Salt of 1-*O*-Palmitoyl-2,2-dimethoxypropane-3-phosphate (VIII)—Three grams of diphenyl chlorophosphate

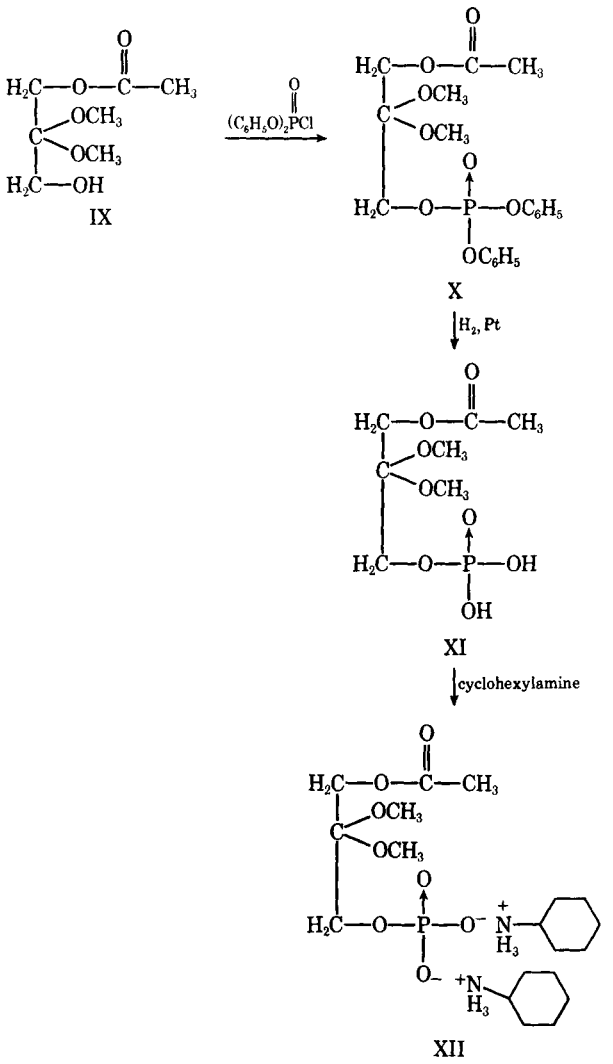


Scheme I—Synthesis of 1-O-palmitoyl-2,2-dimethoxypropane-3-phosphate cyclohexylammonium salt ($R = C_{15}H_{31}$)

was added to a solution of 3.0 g. of Compound V in 15 ml. of pyridine with stirring for 5 min. in an ice bath. The reaction flask was stoppered and kept at 5° for 24 hr. A few drops of water were added to destroy the excess phosphorylating agent, the pyridine was removed under reduced pressure, and the syrupy residue was dissolved in 200 ml. of benzene. The benzene solution was extracted once with 50 ml. of water, twice with 50-ml. portions of 1 *N* HCl, once with 50 ml. of water, twice with 50-ml. portions of saturated sodium bicarbonate solution, and once again with 50 ml. of water, and was finally dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure, and the residue was dried under vacuum, resulting in 4.7 g. of Compound VI.

The phenyl groups were removed by hydrogenolysis. Compound VI was dissolved in 100 ml. of absolute ethanol and was shaken with 0.75 g. of platinum oxide at 15 p.s.i. at room temperature for 3

[1] For analytical purposes and for monitoring column chromatography, TLC was carried out on a 0.25-mm. layer of silica gel G on 5 × 20-cm. glass plates. The plates were activated in an oven at 110°, and the solvents were freshly prepared before use. The components were visualized by charring the plates after they were sprayed with 40% sulfuric acid. Silica gel, 70–325 mesh, was used to prepare columns of 1.9 and 2.7 cm. in diameter. Silica gel G for TLC and silica gel, 70–325 mesh, were purchased from Brinkmann Instruments, Inc. The melting points were taken with a Mel-Temp melting-point apparatus and corrected. Elemental analyses were performed by: Atlantic Microlab, Inc., Atlanta, Ga.; Spang Microanalytical Laboratory, Ann Arbor, Mich.; and Galbraith Laboratories, Inc., Knoxville, Tenn.



Scheme II—Synthesis of 1-O-acetyl-2,2-dimethoxypropane-3-phosphate cyclohexylammonium salt

hr. The catalyst was removed by filtration and washed with ethanol, and 8 g. of cyclohexylamine was added to the filtrate. The mixture was stirred overnight at room temperature and cooled in an ice bath for 1 hr. The white solid was filtered, washed with ice-cold acetone, and dried under vacuum for a few hours. The crude product (3.4 g.) was recrystallized from 90 ml. of an absolute ethanol–acetone mixture (2:1), resulting in 2.7 g. (61%, based on Compound V) of white crystals, m.p. 92–93°.

Anal.—Calc. for $C_{27}H_{56}NO_8P$: C, 58.56; H 10.19; N, 2.52; P, 5.59. Found: C, 58.69; H, 10.27; N, 2.57; P, 5.51.

1-*O*-Acetyl-2,2-dimethoxy-3-hydroxypropane (IX)—This compound was prepared by the procedure described by Ballou and Fischer (18). The boiling point was 68–70° at 0.1 mm. Hg.

Cyclohexylammonium Salt of 1-*O*-Acetyl-2,2-dimethoxypropane-3-phosphate (XII)—The same procedure as described for the preparation for Compound VIII was used with the following exceptions: 5 g. of Compound IX, 11 g. of diphenyl chlorophosphate, 1.5 g. of platinum oxide, and 10 g. of cyclohexylamine were used. After recrystallization from a chloroform–acetone mixture (1:2), 7.6 g. (54%, based on Compound IX) of white crystals was obtained, m.p. 163–165°.

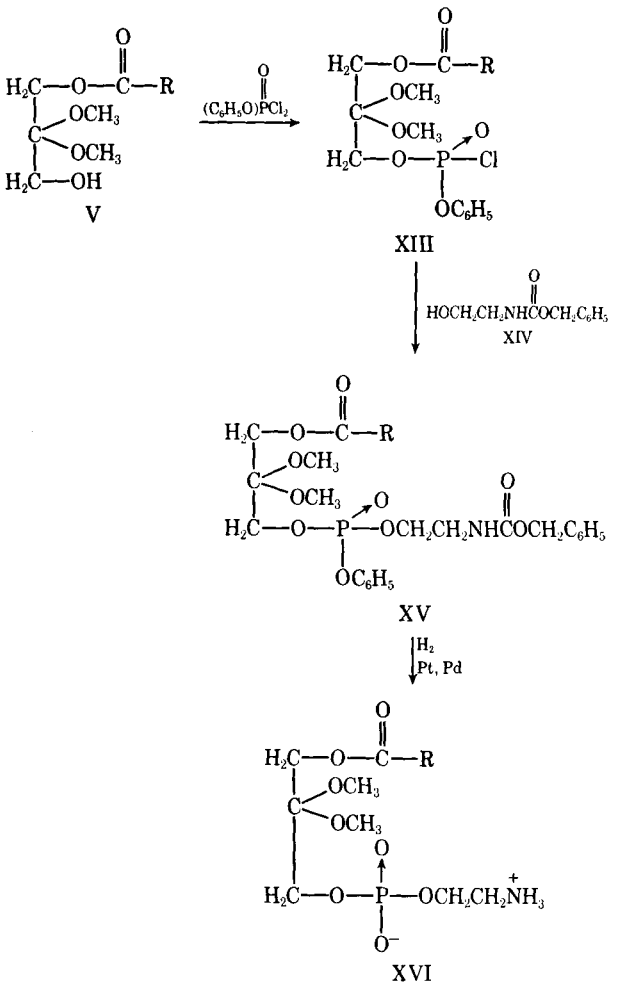
Anal.—Calc. for $C_{19}H_{41}N_2O_8P$: C, 51.26; H, 8.82; N, 5.98; P, 6.61. Found: C, 51.29; H, 8.48; N, 5.71; P, 6.64.

Preparation of 1-*O*-Palmitoyl-2,2-dimethoxypropane-3-*O*-phosphorylethanolamine—The sequence of reactions used is illustrated in Scheme III. Baer *et al.* (21, 22) used a similar procedure in their synthesis of cephalin.

Carbobenzoxyethanolamine (XIV)—This compound was prepared according to the procedure of Rose (23).

1-*O*-Palmitoyl-2,2-dimethoxypropane-3-*O*-phenylphosphoryl-*N*-carbobenzoxyethanolamine (XV)—In a 200-ml. two-necked flask equipped with a magnetic stirrer, dropping funnel, and drying tube was placed 2.5 g. of phenyl phosphorodichloridate in 10 ml. of dry pyridine. The flask was immersed in an ice bath, and a solution of 3 g. of Compound V dissolved in 10 ml. of ethanol-free chloroform was added over a period of 15 min. The mixture was kept for 15 hr. at 5°, the temperature was then raised to 10°, and 2.3 g. of carbobenzoxyethanolamine in 10 ml. of pyridine was added. The mixture was allowed to stand at room temperature for 16 hr. The solvent was removed under reduced pressure, and the viscous residue was dissolved in 300 ml. of ether. The ether solution was washed rapidly with two 100-ml. portions each of 2 *N* H_2SO_4, water, saturated sodium bicarbonate solution, and water. The solution was dried over anhydrous sodium sulfate and evaporated under reduced pressure. The oily crude product was purified with silica gel column chromatography. Four grams of the material was placed on 70 g. of silica gel in a column of 1.9-cm. diameter. The column was eluted with 300-ml. portions of the following solvents: Fraction I, chloroform–benzene (9:1 v/v); Fraction II, chloroform; Fraction III, chloroform–methanol (95:5 v/v). Fraction III contained 2.2 g. of Compound XV, n_D^{24} 1.5028.

1-*O*-Palmitoyl-2,2-dimethoxypropane-3-*O*-phosphorylethanolamine (XVI)—A solution of Compound XV in 100 ml. of absolute ethanol, containing 300 mg. of platinum oxide and 500 mg. of palladium black, was hydrogenated at 15 p.s.i. at room temperature for 3 hr. The catalysts were removed by filtration and washed with 50 ml. of absolute ethanol. The ethanol was removed under reduced pressure, and the white solid product was purified by column chromatography. The material was dissolved in about 5 ml. of chloroform and placed on 80 g. of silica gel in a column of 1.9-cm. diameter. The column was eluted with 300 ml. of chloroform and



Scheme III—Synthesis of 1-O-palmitoyl-2,2-dimethoxypropane-3-O-phosphorylethanolamine ($R = C_{15}H_{31}$)

subsequently with 300-ml. portions of a chloroform–methanol mixture, and the following fractions were collected: Fraction I (9:1), Fraction II (8:2), Fraction III (7:3), and Fraction IV (6:4). Compound XVI was obtained from Fractions III and IV. The solvent was removed under reduced pressure; the white solid product was dissolved in 10 ml. of chloroform and suction filtered. The chloroform was removed under nitrogen, and any residual chloroform was removed under vacuum in a dessicator over phosphorus pentoxide, resulting in 700 mg. of product, m.p. 161–163° (with prior softening). TLC, using solvent systems of chloroform–methanol (8:2 v/v) and chloroform–methanol–water (65:25:4 v/v/v), revealed one spot with R_f values of 0.10 and 0.46, respectively. It gave a positive ninhydrin test.

Anal.—Calc. for $C_{23}H_{48}NO_8P$: C, 55.51; H, 9.72; N, 2.81; P, 6.22. Found: C, 55.17; H, 9.54; N, 2.72; P, 5.99.

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Potential Antihistamines with Increased Receptor Specificity

P. PARIMOO*, B. F. GRABOWSKI▲, and W. G. HANEY, Jr.

Abstract □ A series of *N,N*-dialkylaminoalkyl aniline derivatives were designed and prepared to elicit selective antihistaminic activity. As a result of the relative polarity of these compounds, undesirable CNS, adrenergic, and anticholinergic activities are expected to be less than those of the more lipophilic antihistaminic compounds. The intermediate substituted aniline derivatives were prepared by base-catalyzed substitution of aniline and were then *N*-acylated with 2-bromopropionyl bromide. Characterization of the synthesized compounds was accomplished by elemental analyses and IR and NMR spectroscopy.

Keyphrases □ Antihistamines, potential—synthesis of aniline derivatives for increased receptor specificity □ Aniline, *N,N*-dialkylaminoalkyl derivatives—synthesis, designed to elicit selective antihistaminic activity □ *N*-Alkyl-*N*-[1-(2-bromopropionyl)]aniline derivatives—prepared as selective antihistaminic agents

Many classical medicinal chemical studies have been designed to elucidate drug–receptor interactions. In a typical study (1), a series of compounds is synthesized and evaluated for a particular type of pharmacological activity. Important inferences may be made concerning the nature of the drug–receptor interaction from the structure–activity relationships evolved from such a study. On the basis of these inferences, drugs may be prepared which are assumed to have the characteristics necessary for a strong interaction with the receptor (2).

This approach has been very fruitful and has led to the production of many potent drugs. However, as more active drugs become available in an area of therapy, it becomes apparent that there is not necessarily a relationship between drug potency and drug–receptor specificity. For example, an analgesic compound such as morphine exhibits anticholinergic activity (3), sympatholytic activity (4), *etc.*, in addition to the desired activity. Thus, a potent drug which interacts strongly with the receptor may also interact with other types of receptors and produce side effects.