NEW SYNTHESIS SYSTEM OF (C-11)PROPYL KETENE AND ITS REACTONS WITH VARIOUS ALCOHOLS

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SUMMARY

A new synthesis of (C-11)propyl ketene using a HCl/Helium gas mixture and glass beads was developed. This improved synthesis method gave high yields and good reproducibility, which had been difficult to attain with our previous method. The (C-11) propyl ketene is a useful precursor for labeling, in high specific activity, bioactive compounds which have a hydroxyl group. The reactivity of (C-11)propyl ketene in the acylation of various alcohols was evaluated. The relative reactivity of primary, secondary and tertiary alcohols were 1, 0.4 and 0.1, respectively, and the reactivity of the noncarrier added conditions was similar to that using cold propyl ketene. This (C-11)propyl ketene was also useful for the formation of N-butyl compounds, making it possible to label many bioactive compounds.

Key words : ketene method, C-11 labeling precursor,

INTRODUCTION

The ketene is a high reactive agent reported by Hurd C.D.(1) in 1941. The (C-11)propyl ketene has been reported as a new type of C-11 labeling precursor(2). This precursor may be an effective agent for simple C-11 labeling of useful bioactive materials such as carbohydrate compounds. In our previous studies, the C-11 labeled phorbol esters(3, 4), which are ligands of protein kinase C, make it possible to visualize the in-vivo second messenger and it is useful to elucidate the transmission process between the extracellular signaling and intercelluar signaling. We found a new modified simple (C-11)propyl ketene synthesis technique using a extraction method with an HCl gas mixture instead of the phosphoric acid,

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and using a glass beads as a catalysis of the pyrolysis instead of the glass wool as previously mentioned. Those improvements can produce a good yield and a high specific activity. The (C-11)propyl ketene reactions on various alcohols were evaluated using this technique and discussed the application to the other compounds.

MATERIALS AND METHODS

Chemical materials

Lithium(lump) was purchased from Wako Pure Chemical Industries(Osaka), and n-propyl bromide, 4-dimethylaminopyridine(DMAP), β-phenethyl alcohol, 1-phenyl 2-propanol and 1, 1-dimethyl 2-phenethyl alcohol were purchased from Nakarai Tesque INC.(Kyoto). Phorbol 20-methoxytrityl ether(PMTE) was purchased from LC service corporation(Woburn).

Synthesis of propyl lithium

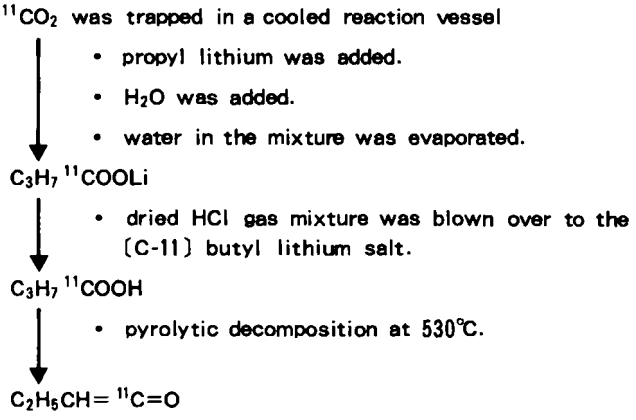
The dried diethyl ether(30ml), lithium(about 5mg) and n-propyl bromide(3.3mmol) were added to the reaction vessel, and the mixture was stirred fast about 30min. at room temperature under dried helium gas. After the reaction, the products were centrifuged and we got pure propyl lithium solution.

Synthesis of (C-11)propyl ketene

We synthesized (C-11)propyl ketene by using the previous method with some modifications. The synthesis steps are shown in Figure 1. The (C-11)CO_2 was produced via $^{14}N(p, \alpha)^{11}C$ reaction using the compact cyclotron(The Japan Steel Works, model BC1710). Initially the reaction vessel was purged by dried helium gas. After the bombardment, the (C-11)CO_2 was trapped in a cooled reaction vessel with liquid nitrogen, and the propyl lithium was added to the reaction vessel. The liquid nitrogen bath was removed from the reaction vessel, and two minutes later the water(100μl) was added to decompose the surplus propyl lithium. The water in the mixture was evaporated completely using vaccum pump at 60℃, and the (C-11)butyl lithium salt was obtained. A quartz glass column(ID : 7mm, length : 240mm) containing the glass beads of 1mm in diameter was heated up at 530℃ beforehand, and the reaction vessel was connected to the column.

A mixture of 0.1% HCl/Helium gas and helium gas, for further dilution, were put through

Figure 1. SYNTHESIS STEPS OF [C-11] PROPYL KETENE



a P_2O_5 column for exclusion of the moisture, and blown over the (C-11)butyl lithium salt. The vapor of (C-11)butyric acid was extracted from the (C-11)butyl lithium salt by HCl, and carried to the quartz glass column by sweep gas. The (C-11)butyric acid was decomposed by pyrolysis at 530℃ to yield the (C-11)propyl ketene. The total flow rate of the gas mixture was 70 ml/min.(flow rate of 0.1% HCl/helium gas was 5 ml/min. and flow rate of helium gas, for further dilution, was 65 ml/min.).

$$^{11}CO_2 \xrightarrow{C_3H_7Li} C_3H_7{}^{11}COOLi \xrightarrow[H^+]{} C_3H_7{}^{11}COOH \xrightarrow[530℃]{} C_2H_5CH={}^{11}C=O$$

It is difficult to analyze the ketene in the native state. Thus we adopted the following method. The yield of the (C-11)propyl ketene was analyzed by acylation of the PMTE as a standard. The PMTE(2 μ mol) and DMAP(1mg) were desolved in dried pyridine(500 μ l), and after the acylation, the (C-11)PMTE was analyzed by HPLC using silica column(Zorbax SIL : ϕ 4mm × 250mm) with eluent of hexane/diethyl etehr/isopropyl alcohol(10:2:1) at a flow rate of 1.8 ml/min.. To evaluate the ketene reaction with various alcohols we selected β-phenethyl alcohol as a primary alcohol, 1-phenyl 2-propanol as a secondary alcohol and 1, 1-dimethyl 2-phenethyl alcohol as a tertiary alcohol. The structure of those alcohols are shown in

Figure 2. Structure of primary, secondary and tertiary alcohols for examination of the [C-11] propyl ketene reactivity.

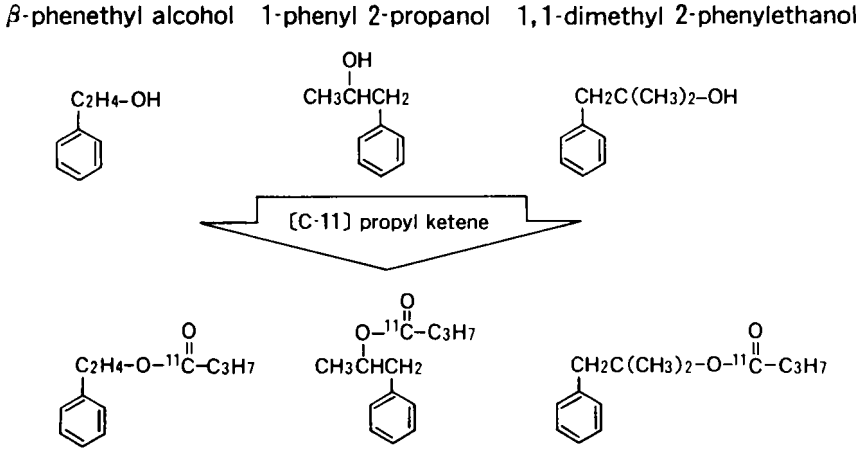


Figure 2. Each alcohol(2 μ mol) was desolved with DMAP(1mg) in dried pyridine, and it was analyzed by HPLC using same column with eluent of hexane/isopropyl alcohol(10:0.5) at a flow rate of 1.8 ml/min.

RESULT AND DISCUSSION

Synthesis of (C-11)propyl ketene

The diagram and the photographic view of the pyrolytic decomposition system are shown in Figure 3 and Figure 4, respectively. The yields of each synthesis steps are shown in Table 1. The total yield of the (C-11)propyl ketene was about 22% and synthesis time was about 25 min. In previous method using phosphoric acid for extraction of the (C-11)butyric acid, it was difficult to obtain the good yield and the efficiency of the pyrolytic decomposition of the (C-11)butyric acid to get (C-11)propyl ketene depended on the condition of the packing of the quartz glass wool. Those problems were resolved by new method in two ways, one of which is the extraction process and the other is the pyrolysis process. As the extraction by the HCl gas mixture was more efficient, the amount of free (C-11)butyric acid were increased, and the efficency of the pyrolytic decomposition was increased by using the glass beads. The combined effect made it possible to get good reproducibility of the (C-11)propyl ketene

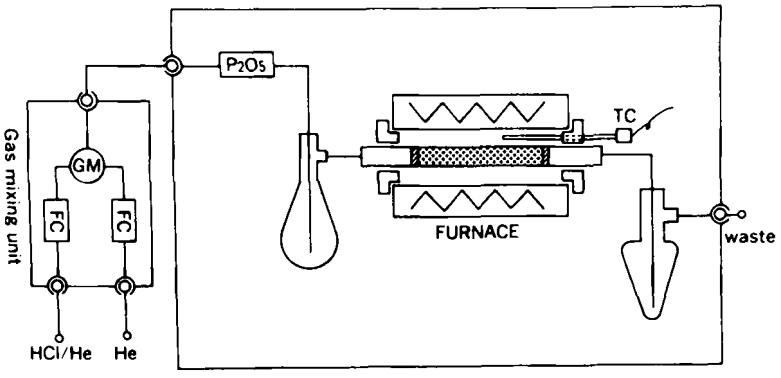


Figure 3. Diagram of the pyrolytic decomposition system

—◎— : connecter, FC : flow controller, GM : gas mixer
▩ : glass beads ▨ : quartz glass wool, TC : thermocouple

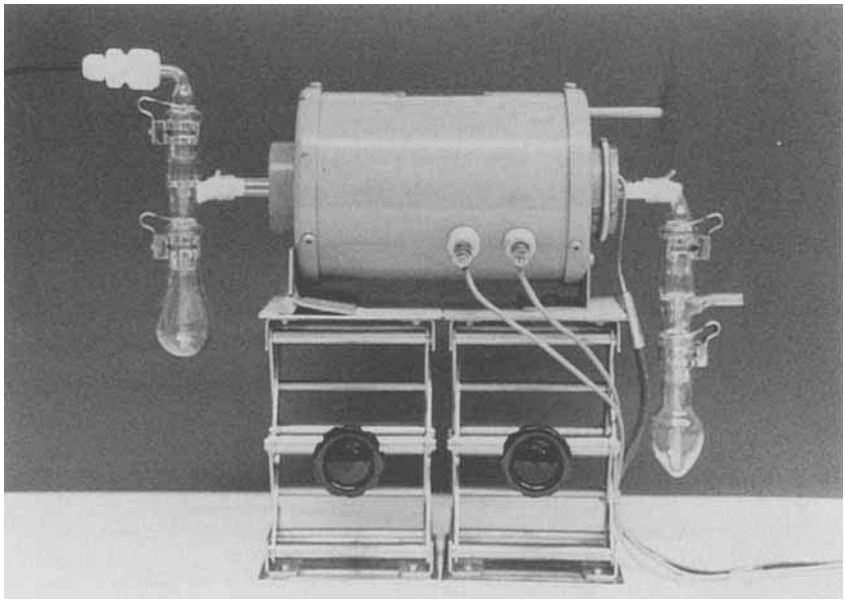


Figure 4. Photographic view of pyrolysis unit

Table 1. Radiochemical yields on each synthesis steps

steps	$C_3H_7Li+^{11}CO_2$	ev. up	pyrolysis	ev. up	final
yield(%)	100	96	32	28	22

Table 2. The reactivity of the (C-11)propyl ketene and the cold propyl ketene to alcohols.

	alcohols		
	primary	secondary	tertialy
$C_2H_5CH=^{11}C=O$	1.0	0.4	0.1
$C_2H_5CH=C=O$	1.0	0.5	0.2

production. In this synthesis method, the concentration of the HCl was very important. In case of low concentration, extrction of the (C-11)butyric acid was incomplete, and it was difficult to get the good reproducibility. And in case of high concentration, the precursor, which is sensitive to acid, may be degraded by surplus HCl. The optimal concentration of this synthesis system was 0.007%.

The propyl ketene reactivity to alcohols

The results of the examination of propyl ketene(labeled and unlabeled) reactivity to alcohols are shown in Table 2. This reaction is driven on the basis of nucleophilic addition of ketene. We examined the reactivity of the unlabeled propyl ketene, which was produced by pyrolytic decomposition of n-butyric acid, to compare the reactivity of each propyl ketenes to alcohols using the same method. The (C-11)propyl ketene reactivity to alcohols, on the basis of presumption that the reactivity to the primary alcohol is 1, the relative reactivity to the

secondary and the tertiary alcohols were 0.4 and 0.1, respectively. The unlabeled propyl ketene reactivity to alcohols, on the basis of the same presumption, the relative reactivity to the secondary and the tertiary alcohols were 0.5 and 0.2, respectively. The reactivity of the ester formation under the non-carrier added condition was similar to that under using cold propyl ketene.

Applicaton to various compounds

As shown in Figure 5, the (C-11)propyl ketene method can be used to get C-11 labeled bioactive compounds, which have very complicated structures such as phorbol esters. Already we successfully synthesized the C-11 labeled various types of phorbor ester(4), diacylglycerol (5) and forskolin for study of the second messengers. Especially phorbol esters are known that it provides a probe for mapping PKC density and permits an evaluation of the second messenger system related to calcium signal employed by a particular neuronal pathway(6). The (C-11)propyl ketene is also useable to produce N-butyryl formation, for example, N-(C-11)butyryl THPO(4, 5, 6, 7 Tetrahydro isoxazoro [4, 5-C]pyridine-3-ol) was synthesized by using (C-11)propyl ketene. This N-butyryl compound may be useable for GABA uptake mechanism. An other C-11 labeled compounds are possible to obtain with high specific activity by using this (C-11)propyl ketene method.

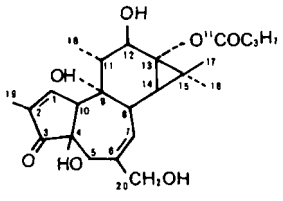
CONCLUSION

By the use of the new synthesis technique, the (C-11)propyl ketene can be obtained reliably with the good yield, and makes it possible to produce an automatic synthesis system. And it is possible to synthesize the other types of ketene which have short or long carbon chain. The (C-11)propyl ketene method can be used to label many compounds which have hydroxyl group or amino groups, and it is very useful to many studies which require a high specific activity. The (C-11)ketene automated synthesis system will be needed in the near future because of those factors above mentioned and necessity of reduction of radiation exposure to the chemist.

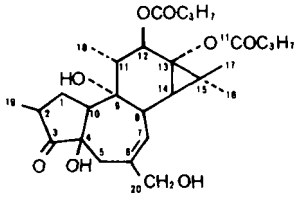
Figure 5. Bioactive Compounds Labeled with (C-11) Propyl ketene

- Phorbol esters*
- Diacylglyserols*
- Forskolin*
- N-butyryl THPO*
- Amines
- Steroids
- Alkaloids
- Carbohydrates

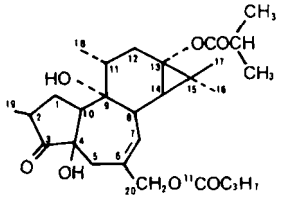
* : Those compounds have been labeled by using (C-11) Propyl ketene.



(C-11) P13Bu

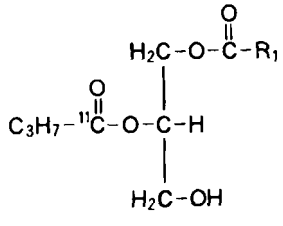


(C-11) PDBu



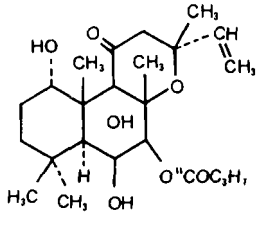
(C-11) DPBB

Structure of the (C-11) Phorbol esters

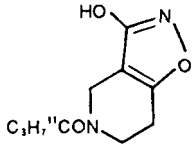


- 1-Palmitoyl 2-(C-11)butyryl glycerol (R_1 : $C_{15}H_{31}$)
- 1-Stearoyl 2-(C-11) butyryl glycerol (R_1 : $C_{17}H_{35}$)

Structure of the (C-11) Diacylglycerols



(C-11) Forskolin



N-(C-11)butyryl THPO

Structure of the (C-11) Forskolin and N-(C-11) butyryl THPO

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