iodine solution was added and kept for 3 min. After raising the temperature to 20°, 1 ml. of the fluorescein solution was added and reacted for 10 min. The temperature was again decreased to 0°, 12.4 ml. of the iodine solution was added and kept for 3 min., and then 8.9 ml. of the fluorescein solution was added and reacted for another 10 min. at 20°. 70.0% of the total activity was found in AcOEt layer.

Discussion

By the Exp. 1 and Exp. 2, it was shown that this deviced method can be applied to prepare sodium radio 3,5-diiodofluorescein ($3,5-{}^{131}I$) to increase the radioactive yield. However, in Exp. 5, where the theoretical maximum yield is 86.5%, the yield was not very much improved. This is mostly owing to the relatively high velocity of the reaction between fluorescein and iodine at room temperature as indicated in Exp. 3. In Exp. 6, the yield was remarkably improved by suppressing the iodination by lowering the temperature during the exchange reaction between iodine isotopes.

Summary

A new method to increase radioactive yield in substitutional reaction is deviced and applied to preparation of sodium radio 3,5-diiodofluorescein ($3,5-{}^{131}I$). In this method, produced radioisotope of inert form is converted into active form by isotopic exchange reaction and reused for the reaction. The radioactive yield was increased from 43% to 70%.

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Gas-liquid Chromatography of Lichen Substances. I. Studies on Zeorin.

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The microchemical techniques have been introduced in lichenology to overcome the difficulty in morphological differentiation which frequently arises among lichens.

The spot color reactions on lichens, the microscopical detection of crystal forms of the extracted constituents and the paper[1~3] and thin-layer chromatographies[4] of the extracts have usefully been applied for such a purpose. Gas-liquid chromatography would also be the most useful tool for chemotaxonomical studies of lichens which can be performed using only a minute sample.

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However, it should be noted that this technique cannot be applied effectively unless the general patterns of gas chromatograms of lichen substances have been established.

A series of studies of this line have been started with a triterpenoid lichen substance, zeorin, which is distributed in various lichen species.

During the process of gas chromatography of triterpenoid, dehydration of the tertiary hydroxyl and rearrangement of the resulting double bond would occur as they have been observed in monoterpene alcohols[5] and some steroids.[6~9]

Zeorin has been known to afford on heating some dehydration products, which would be expected to form on gas chromatogaphy. A comparative study has, therefore, been carried out using zeorin, zeorinone, and their dehydration products.[*3]

Experimental

Samples : Zeorin (I), m.p. 223°,[10] employed in this study was isolated from lichen, *Peltigera dolichorrhiza* Nyl. The other samples described below were prepared from zeorin. Isozeorinin (II), m.p. 185~202°,[11] zeorinin (III), m.p. 179~183°,[11] zeorinone (IV), m.p. 239~245°,[11] isozeorininone (V), m.p. 208~212°,[11] and zeorininone (VI), m.p. 177~178°.[11]

Apparatus——A Shimadzu Model GC-1B gas chromatograph attached with a hydrogen flame ionization detector was employed. The U-shaped stainless steel columns of 2.25 m. length with 4 mm. inner diameter were packed with 0.75% SE-30 on Gas-chrom P (80~100 mesh) or 1.5% SE-30 on Chromosorb W (60~80 mesh).

Trimethylsilylation of Samples——Hexamethyldisilazane (0.1 ml.) and trimethylchlorosilane (0.05 ml.) were added to a solution of samples (1~2 mg.) dissolved in anhydrous pyridine (0.1 ml.). The mixture was shaken vigorously for 30 sec. and allowed to stand for 10 min.

The mixture was centrifuged to precipitate NH_4Cl formed, and the supernatant was injected into the gas chromatograph using a Hamilton microsyringe. In case of employing the samples without trimethylsilylation, they were applied to the gas chromatograph dissolving in acetone or $CHCl_3$.

Results and Discussion

The gas chromatograms developed by two different concentration of stationary liquid phase showed usually sharp peaks. The retention times of 6 compounds are given in Table I.

On employing a column packed with 1.5% SE-30 on Chromosorb W, free zeorin gave two peaks (Fig. 1), while zeorin trimethylsilyl ether revealed three peaks (Fig. 2).

The main peak (t_R=38.7 min.) given by trimethylsilylate of zeorin would be that of its 6,22-bis-trimethylsilyl ether.

The gas chromatograms of zeorin showed a close resemblance with that of isozeorinin, and the minor peak seemed to be identical with the single peak of zeorinin. It is, therefore, reasonably assumed that tertiary hydroxyl at C_{22} of zeorin was dehydrated in high temperature to form an exocyclic double bond which transferred into E-ring. Using 0.75% SE-30 column zeorin trimethylsilyl ether was almost retained without transformation into isozeorinin trimethylsilyl ether, whereas free zeorin was dehydrated to form isozeorinin and in part transformed into zeorinin.

[*3] After the completion of this experiment, we were aware of the appearance of paper reporting similar observation in the gas chromatography of hopane series of compounds including zeorin and zeorinone (N. Ikekawa, S. Natori, H. Ageta, K. Iwata, M. Matsui : This Bulletin, **13**, 320 (1965)).

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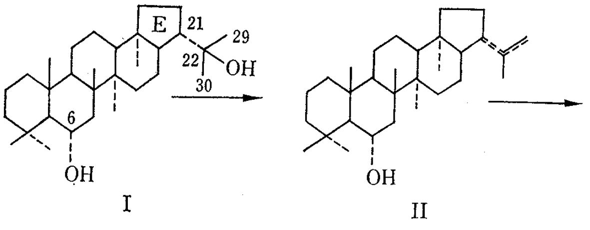
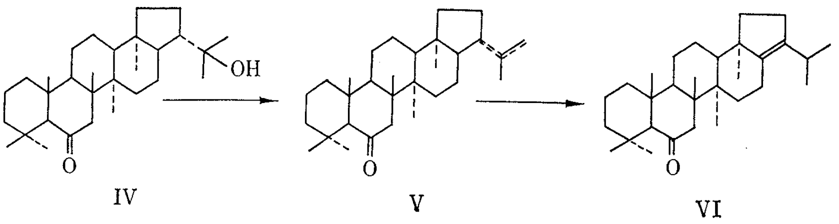
TABLE I. Retention Times (min.) of Zeorin Derivatives in Gas-liquid Chromatography

Compound		0.75% SE-30 on Gas-chrom P (80~100 mesh)	1.5% SE-30 on Chromosorb W (60~80 mesh)
Zeorin (I)	free	4.7(s), 7.3(m)	15.3(s), 24.4(m)
	TMSi[a)]	6.4(s), 11.3(m)	13.5(s), 22.0(s), 38.7(m)
Isozeorinin (II)	free	4.0(s), 7.0(m)	15.2(s), 24.1(m)
	TMSi[a)]	4.1(s), 6.3(s), 7.7(m)	13.3(s), 22.3(m)
Zeorinin (III)	free	4.4	14.6
	TMSi[a)]	4.0	12.9
Zeorinone (IV)	free	4.0(s), 4.5(s), 6.1(m)	12.8(s), 21.0(m)
	TMSi[a)]	4.0(s), 6.3(s), 11.8(m)	12.7(s), 20.4(s), 39.5(m)
Isozeorininone (V)	free	6.1	20.7
Zeorininone (VI)	free	3.8	12.7

a) TMSi: Trimethylsilyl ether
Conditions: Column temp. 240°; flash heater temp. 305°, detector temp. 240°; N_2 flow rate 102.0 ml./min. (0.75% SE-30) and 110.5 ml./min. (1.5% SE-30). m=Main peak, s=Minor peak.





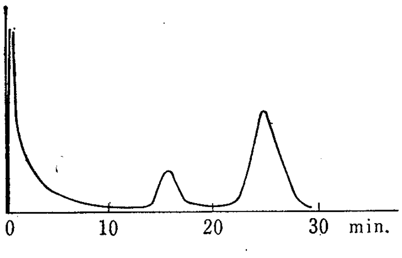


Fig. 1. Gas Chromatogram of Free Zeorin

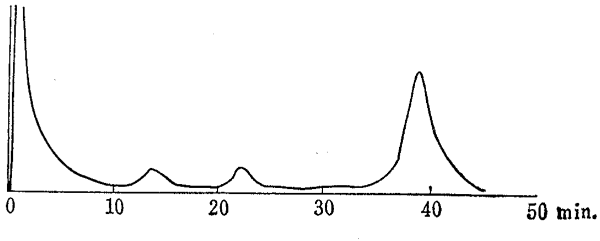


Fig. 2. Gas Chromatogram of Zeorin Trimethylsilyl Ether

Similar results were given in the gas chromatography of zeorinone.

In general, the compound containing endocyclic double bond runs fastest, and the trimethylsilyl ethers run faster than the corresponding free compounds. However, in the case of gas chromatography of isozeorinin trimethylsilyl ether developed on a 0.75% SE-30 column, two peaks appear to show retention times, 6.3 and 7.7 minutes. These peaks show that isozeorinin consist of $\Delta^{21,22}$ and $\Delta^{22(29)}$ compounds, which might be formed on the column duning the process.

We thank Miss Hsüch-Ching Chiang for her co-operation extracting lichen materials.

Summary

The gas-liquid chromatography of zeorin was carried out using free zeorin and its trimethylsilyl ether. The chromatograms were analyzed to discuss the transformation of the compounds during the process.

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