mixture of the isomers). Together with our previous finding that addition of alcohol occurs on irradiation,[4a] it has now been established that the imide carbonyl of **1** is capable of photochemically adding the methylene (or methine) adjacent to oxygen. In contrast to the well-known corresponding reactions of ketones, none of pinacol formation has been observed.

On irradiation with a 100 W high-pressure mercury lamp (pyrex filter) for 8 hr in acetonitrile solution containing olefin such as cyclopentene and cyclohexene (2.0M), **1** (30 mM) underwent similar addition to afford carbinols (**6** (3%, 74% sm) and **7** (10%, 70% sm)). It is again worthy to note that no corresponding oxetanes have so far been isolated. In conclusion, the major photoreactions of the phthalimide system in the presence of ether, alcohol, and olefin are the carbinol formation (eq. 1). Synthetic scope and mechanism of the reactions are under investigation.

Faculty of Pharmaceutical Sciences,
Hokkaido University,
Kita 12 jo, Nishi 6 chome, Kitaku,
Sapporo, 060, Japan

YUICHI KANAOKA
YASUMARU HATANAKA

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Beta Unsubstituted Cyclopentenone, a Structural Requirement for Antimicrobial and Cytotoxic Activities[1]

The recent report[2] of two new antibiotics, pentenomycins I and II (I and II), which possess a beta unsubstituted cyclopentenone ring system by Umino and his co-workers[3] prompts this communication of our work.

During the course of an investigation for the relationship between the sesquiterpene lactone structure and the antitumor/cytotoxic activity,[4] we had also screened a series of helenalin (III) related derivatives[5] for the antimicrobial activity according to Mitscher's method.[6] A comparison of the values for the minimum inhibitory concentration of these compounds suggests that a beta unsubstituted cyclopentenone ring moiety such as in helenalin (III) and plenolin (IV)[7] (Table I) contributes to the antimicrobial activity. The corresponding saturated compounds (V and VI) gave a more than 10-fold decrease in activity. The significant antimicrobial activity appears to be independent of the presence or absence of an α-methylene-γ-lactone moiety (compare compounds III with IV and V with VI). A more than 10-fold diminution in antimicrobial activity was also observed when the beta position of the cyclopentenone ring was substituted such as in deacetoxymatricarin (VII)[8] and compound IX

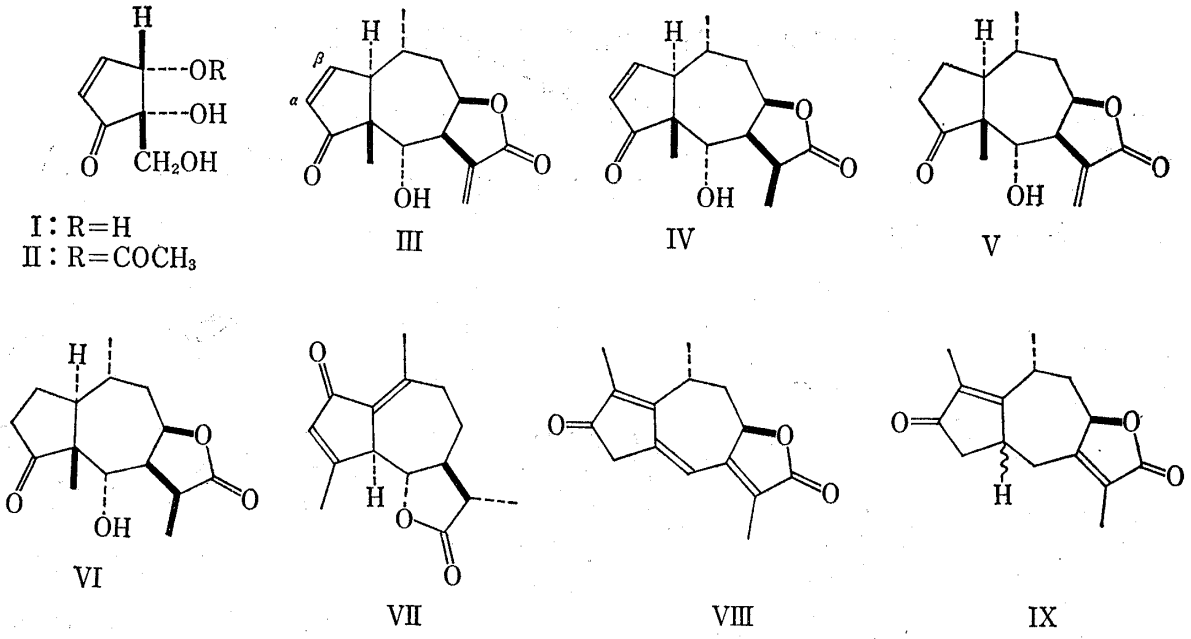
1) Previous paper in this series: Antitumor Agents VIII, K.H. Lee, S.H. Kim, C. Piantadosi, E.S. Huang and T.A. Geissman, *J. Pharm. Sci.*, in press.
2) This report came to the authors' attention after the completion of their work.
3) K. Umino, T. Furumai, N. Matsuzawa, Y. Awataguchi, Y. Ito and T. Okuda, *J. Antibiotics* (*Tokyo*), *Ser. A.*, **26**, 506 (1973).
4) K.H. Lee, *J. Pharm. Sci.*, **62**, 1028 (1973); and references cited therein.
5) Over 30 derivatives had been screened and their biological data will be discussed in detail elsewhere.
6) L.A. Mitscher, R.P. Leu, M.S. Bathala, W.N. Wu and J.L. Beal, *Lloydia*, **35**, 157 (1972).
7) K.H. Lee, T. Ibuka, A.T. McPhail, K.D. Onan, T.A. Geissman and T.G. Waddell, *Tetrahedron Letters*, **1974**, 1149.
8) T.A. Geissman, T. Stewart and M.A. Irwin, *Phytochemistry*, **6**, 901 (1967).

(mp 181—182°) which was synthesized[9] from helenalin (III) *via* intermediate VIII (mp 161—163°) although both compounds VIII and IX possess a somewhat different structural type in comparison with those of helenalin (III) or plenolin (IV). Further investigations are in progress which are aimed at evaluation of the significance of the beta unsubstituted cyclopentenone for potential antimicrobial activity.

Table I. Antimicrobial and Cytotoxic Activities of Helenalin Related Derivatives and Sesquiterpene Lactones

Compound	M.I.C.[a] (mcg/ml)	ED_{50}[b] (mcg/ml) (H. Ep.-2)[c]
Helenalin (III)[4,10,11]	100	0.10
Plenolin (IV)[7,10]	100	0.81
2,3-Dihydrohelenalin (V)[10]	>1000	3.84
Tetrahydrohelenalin (VI)[10]	>1000	>40
Deacetoxymatricarin (VII)[8,d]	>1000	>20
Compound (VIII)[4]	>1000	>20
Compound (IX)[4]	>1000	>20

a) Minimum inhibitory concentration against *Staphylococcus aureaus* PS 80—81 and *Bacillus subtilis* PCI 219.
b) ED_{50} values were determined according to a rapid microtiter method.[e]
c) H. Ep. -2 refers to human epidermoid carcinoma of the larynx.[d]
d) K. H. Lee, E.S. Huang, C. Piantadosi, J. S. Pagano and T.A. Geissman, *Cancer Res.*, **31**, 1649 (1971)
e) E.S. Huang, K.H. Lee, C. Piantadosi, T.A. Geissman and J.S. Pagano, *J. Pharm. Sci.*, **61**, 1960 (1972)



Previously we had indicated[10,11] that one of the structural requirements for significant cytotoxicity is a cyclopentenone ring system as the major active center such as in helenalin (III) and plenolin (IV). It should be noted, however, that this cyclopentenone could be only limited to a beta unsubstituted ring system, *i.e.* the introduction of a beta substituent into the cyclopentenone ring system could destroy its cytotoxicity. This was verified by the

9) K.H. Lee and T. Ibuka, unpublished data.
10) K.H. Lee, H. Furukawa and E.S. Huang, *J. Med. Chem.*, **15**, 609 (1972).
11) K.H. Lee, R. Meck, C. Piantadosi and E.S. Huang, *J. Med. Chem.*, **16**, 299 (1973).

fact that the compounds VII, VIII and IX were essentially inactive in comparison with the ED_{50} values for the cytotoxicities of III and IV (*cf.* Table I).

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Department of Medicinal Chemistry
School of Pharmacy
University of North Carolina
Chapel Hill, North Carolina 27514
U.S.A.

KUO-HSIUNG LEE
TOSHIRO IBUKA

Institute of Botany
Academia Sinica
Nankang, Taipei, Taiwan
Republic of China

RONG-YANG WU

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Spectrophotometric Determination of Bunte Salts[1)]

S-Alkylthiosulfates and S-arylthiosulfates, known as "Bunte salts," have attracted particularly wide interest in the textile and pharmaceutical industries.[2)] Some of them are also reported to exhibit various types of biological activity.[2)] In spite of the industrial and biological significance, any general method of determination of Bunte salts has not been reported.

During the course of our investigation on the bacterial metabolism of Bunte salts, development of a microanalytical method of Bunte salts in biological materials was required. In this communication, a colorimetric and a fluorometric methods of determination of Bunte salts are described.

We first applied the Cleland's reagent (DTT),[3)] which is known to reduce disulfide bonds, to the reduction of Bunte salts. The reduction of $CySSO_3H$ and $(PaSSO_3)_2Ca$ (Fig. 1) as well as $CyNSSO_3H$, $DMCyNSSO_3H$, $BzSSO_3Na$, $PATPSSO_3H$ and $(P\text{-}PaSSO_3)_2Ca$ proceeded rapidly and quantitatively in alkaline media. $PeSSO_3H$ was not reduced under the same condition probably because of its steric hindrance around the $-S-SO_3^-$ group. An equimolar reaction of $CySSO_3H$ with DTT was confirmed by the continuous variation method (Fig. 2) and the molar ratio method.

1) The abbreviations used are: DTT, dithiothreitol; $CySSO_3H$, S-sulphocysteine; $(PaSSO_3)_2Ca$, calcium salt of pantetheine-S-sulfonic acid; $(P\text{-}PaSSO_3)_2Ca$, calcium salt of 4′-phosphopantetheine-S-sulfonic acid; $CyNSSO_3H$, cysteamine-S-sulfate; $DMCyNSSO_3H$, dimethylaminoethanethiol-S-sulfate; $PeSSO_3H$, penicillamine-S-sulfate; $BzSSO_3Na$, benzylmercaptan-S-sulfate sodium salt, $PATPSSO_3H$, *p*-aminothiophenol-S-sulfate; NEM, N-ethylmaleimide; CySH, L-cysteine; EDTA, ethylenediaminetetraacetic acid disodium salt; PCMB, *p*-chloromercuribenzoic acid.

2) H. Distler, *Angew. Chem. Int. Ed. Engl.*, **6**, 544 (1967); S. Oae, G. Tsukamoto, and T. Kurusu, *Kagaku* (Kyoto), **26**, 1066 (1971).

3) W.W. Cleland, *Biochemistry*, **3**, 480 (1964).