difference in sedimentation was small, we performed a mixing experiment in which nuclear extracts containing unprocessed and processed ER labeled with ^3H or ^{125}I were mixed and sedimented together. Receptor treated for 6 hours with E_2 sedimented more rapidly than that treated for 30 minutes (Fig. 4b). We also reacted nuclear ER with a monoclonal antibody (D547Spγ) to receptor from MCF-7 cells (*7*) and analyzed the receptor-antibody complexes on sucrose density gradients. With both receptor forms, the receptor-antibody complexes were shifted to 8.8*S* (Fig. 4c), suggesting that in both cases E_2 was bound to putative ER. We conclude that the physical changes reported occurred in ER and do not represent transfer of the ligand to some alternative nuclear moiety. Receptor ionic properties during processing were further examined by HAP chromatography. Most of the ER labeled for 30 minutes eluted as a single peak at 170 to 180 m*M* potassium phosphate (Fig. 5). In contrast, processed receptor gave two peaks, one at 180 m*M* and one at 280 m*M* potassium phosphate. Peak fractions from both receptor forms showed appreciable (19 to 26 percent) binding to DNA-cellulose (data not shown).

We have thus described a hormone-inducible, intranuclear receptor-processing event (or events) that results in the transformation of nuclear ER to a less exchangeable, slowly dissociating form of receptor. This receptor form is separable from remaining nuclear ER by sucrose gradients and HAP chromatography. While most models of estrogen action are based on obligatory transformation of receptor from 4*S* to 5*S* for mediation of nuclear entry, our results in conjunction with those of other studies (*8*) show that, at least in MCF-7 cells, this transformation is a nuclear process. Further, we have observed a contemporaneous nuclear event associated with receptor processing. After 5 to 6 hours of perfusion with E_2 a rapidly dissociating population of ER is generated, with a subsequent true loss of binding associated exclusively with these sites (*9*). The question now arises as to which of these processing events takes part in initiating hormone action. We believe that true ER loss and receptor modifications may occur simultaneously during processing, since the two events are temporally related. While the quantitative loss of ER is variable and dependent on a variety of growth factors, estrogen responsiveness (as manifested by the induction of PR) is equivalent under all conditions. Therefore we suggest, as have others (*10*), that there is no direct correlation between the extent of ER processing and PR induction in MCF-7 cells. Further, in rat uterus the total concentration of cellular ER is quantitatively conserved after continuous availability of E_2 to rats for 6 hours; within the same period, hormone-induced biochemical and immunological changes occur in both cytoplasmic and nuclear receptor (*11*). Thus, quantitative changes in receptor content may not be directly related to the onset of hormone action. The changes in molecular characteristics of nuclear ER that we report are invariant and independent of receptor loss. Elucidation of the functional significance of these receptor modifications in eliciting specific changes in gene expression will require further experimentation.

Table 2. Effect of assay conditions on concentrations of nuclear estradiol-receptor complexes (ER). MCF-7 cells were treated with 3 n*M* [^3H]E_2 (direct assay) or 3 n*M* unlabeled E_2 (exchange assay) for 1 or 6 hours under competing conditions at 37°C. Cells were harvested and washed, and nuclear extracts were prepared by either sonication or Dounce homogenization. Extracted receptor was bound to HAP and measured in two ways. In the direct-binding assay, the amount of specific ER was determined by subtracting nonspecific binding from the total binding. In the exchange assay, ER bound to HAP was incubated at 30°C for 3 hours with 3 n*M* [^3H]E_2 plus 300 n*M* diethylstilbestrol to estimate the degree of nonspecific binding.

Time of hormone exposure (hours)	Assay technique	Receptor concentration (picomoles per milligram of DNA)
	Dounce homogenization	
1	Direct	1.61
6	Direct	1.16
	Sonication	
1	Direct	1.98
6	Direct	2.02
1	Direct	4.9
6	Direct	4.6
1	Exchange	3.9
6	Exchange	2.1

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Bimolane: Structure Determination Indicates Anticancer Activity Is Attributable to ICRF-154

Abstract. *X-ray diffraction studies of crystals from samples of bimolane synthesized in China and in the United States showed that the crystals consist of the related compound ICRF-154. Analysis of the results of biological tests did not show any significant differences between the anticancer activity of bimolane and ICRF-154. It appears that the anticancer activity of bimolane is due to ICRF-154.*

The synthetic diketopiperazine derivative bimolane (Fig. 1a) has been reported to have a high chemotherapeutic index against various malignant tumors in lengthy clinical trials in China (*1*). These results have generated considerable interest (*2*), culminating in contractual syntheses, in vitro testing, and wide distribution of bimolane for biological tests by the National Cancer Institute (NSC 351358). We have been interested in correlating stereochemistry and biological efficacy in diketopiperazines (*3*) and have investigated the structure of crystals obtained from samples of bimolane. The structure determination showed the

crystals to contain ICRF-154 (Fig. 1b), not bimolane, and indicates that ICRF-154 may be the active agent in test results attributed to bimolane.

Two samples of bimolane, one (sample NA) synthesized in China and the other (NB) in the United States, were received from the Drug Synthesis and Chemistry Branch, National Cancer Institute. Crystals were obtained from both samples by solvent evaporation in dimethyl sulfoxide, in 95 percent ethanol, and in mixtures of acetone and water; all crystals gave identical x-ray diffraction patterns. Crystal data are as follows: monoclinic, $a = 5.633$ Å, $b = 16.786$ Å, $c = 7.044$ Å, β (angle between a and c) = 122.37°, and space group $P2_1/c$, with two molecules in the unit cell (one-half molecule composing the asymmetric unit). Density considerations indicated that the crystals could not contain molecules the size of bimolane, and we undertook a structure determination to ascertain the composition of the crystals.

X-ray diffraction data were collected on an automated four-circle diffractometer with Cu radiation; there were 713 observed maxima [intensity (I) > $3\sigma(I)$] in the range $0 < 2\theta$ (dispersion angle) < 130°. The structure was solved by direct phasing procedures and refined by anisotropic full-matrix least squares to a final discrepancy index of 0.046. The results are shown in Fig. 2: the molecular structure is that of 1,2-bis(3,5-dioxopiperazin-1-yl)ethane (ICRF-154), a structural component of bimolane and a substance which has been shown previously to possess antitumor activity (*4*).

It was important to determine whether the ICRF-154 crystals were a consequence of bimolane decomposition during the crystallization procedures or whether the presence of ICRF-154 is an inherent property of the samples. The latter possibility is suggested by the identity of the crystals obtained from various solvent systems under different conditions and by melting point data (213° to 217°C for sample NA and 198° to 203°C for sample NB, compared to 236° to 239°C quoted on the product information sheet from NCI). Subsequently, 300-MHz nuclear magnetic resonance data obtained for these samples and others by an independent contractor were interpreted as indicating that all samples contained 33 to 40 percent impurities (*5*).

Evaluation of bimolane for anticancer activity by the NCI tumor panel indicated high activity against B16 melanoma and L1210 leukemia and moderate activity against P388 leukemia. On being notified of our structural results, NCI ascertained that ICRF-154 had been tested previously (NSC 129942) in similar systems, and had demonstrated activity very similar to that of bimolane in the B16 melanoma and L1210 and P388 leukemia tests (results in all cases were within the range of experimental error) (*5*).

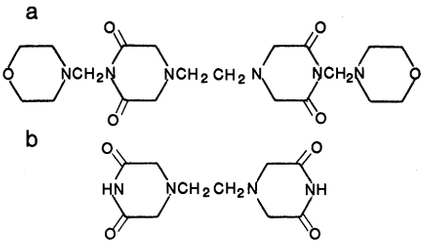

Fig. 1. Molecular structure of bimolane (a) and of ICRF-154 (b).

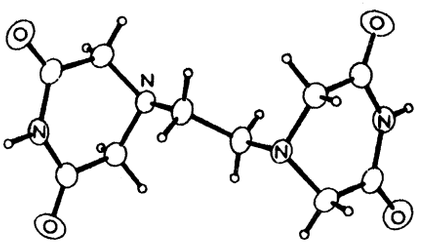

Fig. 2. Molecular structure of ICRF-154 crystallized from bimolane samples. Unlabeled atoms are C and H.

Thus the structural, chemical, and biological data strongly suggest that bimolane is inherently unstable and that the antitumor activity ascribed to it may be attributable to ICRF-154. In addition, since bimolane has been reported to be effective in the treatment of psoriasis and uveitis (*1*), it may be useful to test ICRF-154 for chemotherapeutic activity against these conditions.

The conformational characteristics and molecular parameters of ICRF-154 are similar to those observed in other diketopiperazines we have structurally elucidated (*3*). Structural parameters for the compound are available (*6*).

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Cell Surface Changes Associated with Cellular Immune Reactions in *Drosophila*

Abstract. *In* Drosophila melanogaster *a temperature-induced change in immune competence accompanies cell surface alterations that cause its blood cells to adhere and to encapsulate a parasite. At 29°C the blood cells of the tumorous-lethal* (Tum^l) *mutant show a high degree of immune competence and encapsulate the eggs of the parasitic wasp* Leptopilina heterotoma. *At 21°C the blood cells are essentially immune incompetent. High percentages of lectin binding cells were found under conditions which potentiated cellular encapsulation responses. Some immune reactive blood cells did not bind lectin. The low percentages of lectin binding cells in susceptible hosts suggest that developing parasites alter the cell surface of the blood cells of immune reactive hosts.*

Unlike vertebrates, insects and other invertebrates lack immunoglobulin-like recognition molecules and yet manifest a high degree of specificity in their immune responses against a diversity of nonself components (*1*). Unfortunately, little is understood of the mechanisms of nonself recognition in insects. This kind of information, which is available for only a few insect species, is important since many disease causing agents that debilitate humans live for various periods of time in the body cavities of their insect vectors. For these and other endoparasites to develop unmolested within potentially immunologically hostile environments, they must be able to evade detection or actively suppress the defense mechanisms of their hosts (*2*). A basic understanding of how hosts identify nonself components requires knowledge of the types of immune competent