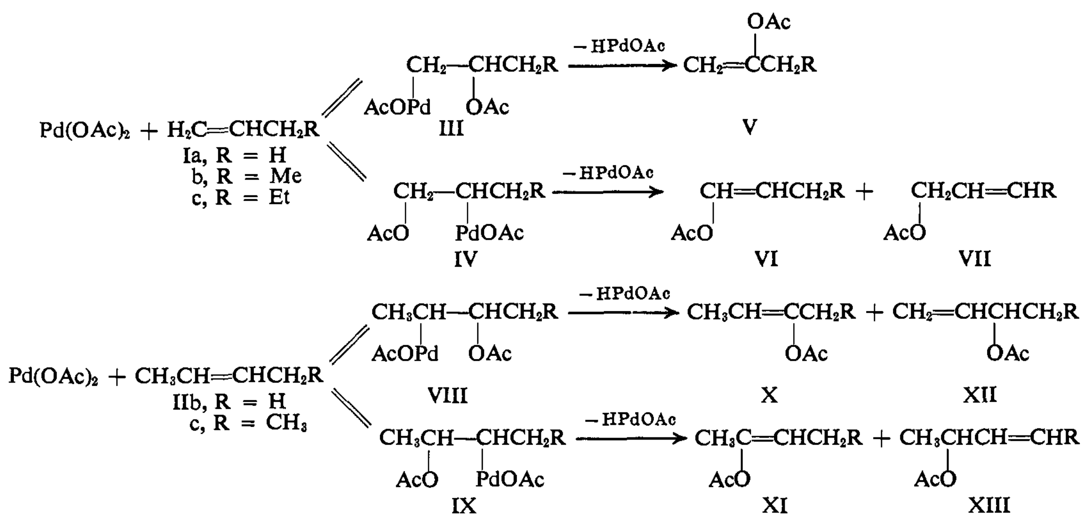
Scheme I



secondary isomer^{7b} XIIb. Similarly, *cis*-2-pentene leads very predominantly (>92%) to allylic acetate which is exclusively (>99.5%) *secondary*, 70% isomer^{7b} XIIc and 30% XIIIc^{7b} being formed.

The above product distributions are from early observations in order to obtain values close to kinetic control proportions. After long reaction times, product isomerizations are evident. For example, allylic isomerization of the allylic acetates occurs, and it was demonstrated separately that primary and secondary allylic acetates, *e.g.*, VIIb and XIIb, are equilibrated by $Pd(OAc)_2$ in AcOH, with concurrent material loss due to other reactions.

As regards allylic ester product, the $Pd(OAc)_2$ oxidation contrasts sharply with its $Hg(OAc)_2$ counterpart.[2] While the latter oxidation gives pure secondary allylic acetate from both 1- and 2-olefins, $Pd(OAc)_2$ gives a separate allylic acetate from each olefin. The nature of the products from the $Pd(OAc)_2$ oxidation and mechanistic considerations cause us to rule out "frozen" or "switching" σ-allylpalladium species and also π-allylpalladium counterparts as significant intermediates. An actual study of the rates and products of acetolysis of π-crotylpalladium acetate confirms that it can be ruled out as an intermediate in the $Pd(OAc)_2$ oxidation of any of the butenes.[10]

The only mechanism for the present $Pd(OAc)_2$ olefin oxidations which appears to accommodate the facts involves oxypalladation,[4] presumably reversible, of the olefin to give the adducts[11] III, IV, VIII, and IX. These eliminate the elements of HPdOAc[12] to yield either enol acetate or allylic acetate depending on the direction of elimination. On this basis, the available data are rationalized in a general way if (i) Markovnikov addition is preferred over non-Markovnikov in oxypalladation, and (ii) elimination to give allylic acetate tends to be preferred over the alternative one leading to enol acetate. This rationalization still leaves the details of stereochemical, conformational, and substituent effects to be clarified before the exact product distributions are more completely understood. Also not clear are solvent or ligand effects on the course of the oxidations. For example, when the $Pd(OAc)_2$ oxidation of 1-butene is performed in a 70 vol. % dimethyl sulfoxide–30% AcOH mixture instead of AcOH, the proportion of allylic acetate, still exclusively primary, in the product rises from the previous 9% to *ca.* 73%.

While oxymercuration–deoxymercuration is incidental to the $Hg(OAc)_2$ allylic oxidation, oxypalladation plays an essential role in the $Pd(OAc)_2$–AcOH allylic oxidation. Reversible oxypalladation–deoxypalladation appears also to provide a mechanism for the allylic isomerization of the allylic esters.[13] Actual comparisons show $Pd(OAc)_2$ in AcOH to be considerably more efficient in this respect than is $Hg(OAc)_2$.

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[10] W. Kitching and Z. Rappoport, unpublished work.

[11] These are written as monomeric only for simplicity, and not to reflect any information on the actual state of aggregation.

[12] The intimate mechanism here and the mode of formation of the final Pd° are not clear. See E. H. Brooks and F. Glockling, *Chem. Commun.*, 510 (1965), for a recent report of the phosphine-stabilized $HPd(PEt_3)_3Cl$.

[13] This type of mechanism seems to apply also to PdX_2-catalyzed *trans* esterification of vinylic esters.[3]

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Received March 21, 1966

Adsorption of Nitrogen on Ice at 78°K

Sir:

We are currently investigating the physical adsorption of vapors on low-melting adsorbents, *i.e.*, nonionic small molecule substances. Ice is an interesting member of this class and one for which, curiously, no adsorption studies appear to have been published. Possibly related to the present results, however, are those of Karasz, *et al.*,[1] for nitrogen adsorption on TiO_2 layered with preadsorbed water.

The ice samples were prepared by condensing water vapor, in the absence of foreign gases, onto a liquid nitrogen cooled glass surface. This was done either *in situ*, using the sample bulb with which the adsorption isotherm was then determined by standard gas volumetric procedure, or in a large flask with the sample

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[1] F. E. Karasz, W. M. Champion, and G. D. Halsey, Jr., *J. Phys. Chem.*, **60**, 376 (1956).

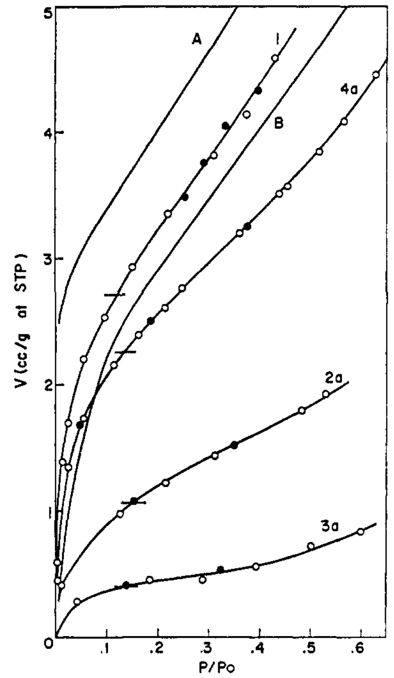

Figure 1. Adsorption of nitrogen on ice at 78°K. Shaded points are for desorption. Curves marked A and B are for adsorption on TiO_2 (ref 5) and polyethylene (ref 4) on an arbitrary *v* scale. Shaded points are for desorption. Horizontal lines indicate BET V_m values.

then transferred to the bulb as a slurry in liquid nitrogen. All data are for nitrogen adsorption at 78°K, but, as indicated in Table I, some samples were rerun after a period of annealing at a higher temperature. The isotherms are shown in Figures 1 and 2; they were reversible with respect to desorption and reproducible in that original (nonannealed) samples, held overnight at 78°K, yielded the same isotherm as that determined after preparation. However, different preparations differed, apparently because of variations in ice-deposition rates; empirically, BET *c* values decreased with decreasing specific surface area.

Table I. Adsorption of Nitrogen on Ice at 78°K

	Sample	BET constants		
Run	wt, g	Σ^a	*c*	Conditions
1	3.026	11.8	70	Transferred as slurry
2a	5.477	4.5	39	*In situ* preparation
2b	5.477	<0.2	. .	Overnight at −70°
3a	8.693	1.8	49	Transferred as slurry
3b	8.693	0.40	20	30 min at −80°
4a	6.839	9.8	52	Transferred as slurry
4b	6.839	0.35	8	Allowed to warm to −50° over a period of 3 hr

[a] Based on 16.2 A² for N_2.

The data suggest two conclusions of potential interest. First, the structures of ice, particularly with respect to the locations of hydrogen atoms, constitute a subject of notorious difficulty.[2] A related matter is whether the surface of ice is, at the one extreme, highly polar with a preponderance of exposed oxygen atoms, or, at the other extreme, nonpolar with primarily hydrogen atoms exposed. Our results suggest the second condition; in particular BET *c* values of 17 and 6 for runs 3b and 4b, as well as the general isotherm shapes, suggest a surface akin to that of a nonpolar solid such as polytetrafluoroethylene.[3] Such inert character suggests that hydrogen atoms form the outer layer and, moreover, that they are not in the relatively polar form of hydroxyl groups. Possibly, each hydrogen bridges two oxygen atoms.

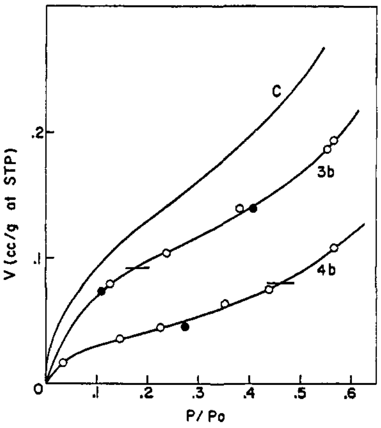

Figure 2. Adsorption on annealed samples. Curve C is for adsorption on polytetrafluoroethylene (ref. 3).

Even original samples, while more polar than annealed ones, gave isotherms close to that for polyethylene[4] and quite different from that for a polar solid such as TiO_2.[5] Also, isotherm 4a included points down to $\theta = 0.05$, and the submonolayer portion fitted the Langmuir isotherm well enough to suggest that the surface was relatively homogeneous. Interestingly, the nitrogen isotherm reported[1] for anatase, having a nominal 12 layers of predeposited water, is very similar to that of run 4a although the circumstances and temperatures of preparation of the surface are quite different.

Second, the annealing effect is of interest. Ice formed under our conditions is very likely amorphous and should transform into cubic ice at about −120° and into ordinary ice I at about −87°.[6] Runs 1, 2a, 3a, and 4a are therefore probably for the amorphous form, and runs 2b, 3b, and 4b, for ice I. Yet a change in crystal structure should, if anything, be accompanied by an *increase* in surface area due to some shattering of crystallites, while we observe a drastic *reduction.* Moreover, the reduction does not seem to be assignable to sintering as the annealing was not accompanied by any change in the appearance of the brightly white powder or in its apparent density. It is more consistent with our observations to consider that the original samples consisted of relatively large particles with very rough, brush-heap surfaces, and that around −70° there was a rapid smoothing due to surface migration.

Some measurements were made on a snow sample. This was taken from falling snow powder (Dec 21, 1965, 8000-ft level of Mt. San Gorgonio) showing clusters of blunt needles and flakes of the type depicted by

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(2) See P. G. Owston, *Advan. Phys.*, 7, 171 (1958).

(3) D. Graham, *J. Phys. Chem.*, **66**, 1815 (1962).

(4) A. C. Zettlemoyer, *J. Am. Chem. Soc.*, 72, 2752 (1950).

(5) A. W. Adamson and I. Ling, Advances in Chemistry Series, No. 33, American Chemical Society, Washington, D. C., 1961, p 51.

(6) See J. A. McMillan and C. S. Los, *Nature*, **206**, 806 (1965).

Bentley and Humphreys.[7] The specific surface area was only about 0.2 m²/g, or about the geometric expectation, and the isotherm, while not accurately determinable, appeared similar to that of run 4b. A second sample of 11.3 g of day-old Denver snow, collected on Jan 18, 1966, gave reproducible isotherms corresponding to the very low c value of unity and a Σ of 0.4 m²/g. Surface contamination from adsorption of atmospheric impurities cannot be excluded in this case, however.

Acknowledgment. These studies have been supported in part by the Petroleum Research Foundation.

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(7) W. A. Bentley and W. J. Humphreys, "Snow Crystals," Dover Publications, New York, N. Y., 1962, p 170.

(8) Stauffer Fellow, 1965–1966.

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Recieved January 7, 1966

A Procedure for the Selective Modification of Carboxyl Groups in Proteins

Sir:

The chemical modification of amino acid residues provides essential information in the correlation of protein structure and function. There is at present no simple published procedure for producing well-characterized modifications of carboxyl groups which is capable of general application to proteins. Methanolic solutions of HCl can give good yields of methyl esters, but the high methanol and HCl concentrations limit their use to the most stable proteins.[1,2] Much milder conditions may be used with diazo compounds, but the instability of these reagents in aqueous solution severely limits the yields of esters which can be obtained.[3-8] Carbodiimides have also been used[9-12] as modifying reagents under mild conditions, but the products are poorly characterized, or the yields are low.

We have found that a procedure involving a water-soluble carbodiimide and a modifying reagent can lead to rapid and quantitative modification of carboxyl groups under mild conditions. The basic reaction is shown in eq 1, in which the carboxyl group is activated by the carbodiimide in the first step, presumably by the mechanism suggested by Khorana.[13] The nucleophilic modifying reagent, HX, then attacks the activated carboxyl group to yield the products. A typical procedure involves N-benzyl-N′-3-dimethylaminopropylcarbodiimide (BDC) as the activating carbodiimide and glycine methyl ester as the modifying reagent. BDC was synthesized from benzyl isocyanate and N,N-dimethyl-1,3-propanediamine by the method of Sheehan and coworkers.[14] It was crystallized from methylene dichloride–ether mixtures as its *p*-toluenesulfonate, mp 118–119°). *Anal.* Calcd: C, 61.7; H, 6.94; N, 10.80; S, 8.23. Found: C, 61.7; H, 6.79; N, 11.03; S, 8.37.

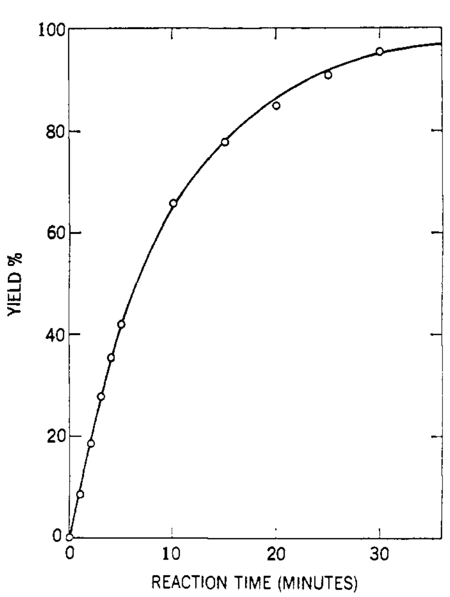
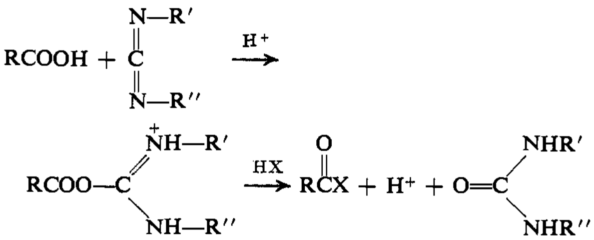


Figure 1. The yield of methyl *m*-nitrohippurate from the reaction of *m*-nitrobenzoic acid (5×10^{-3} *M*) and glycine methyl ester (1.0 *M*) with BDC (0.10 *M*) at 25° and pH 4.75 maintained with a pH-Stat.



$$\mathrm{RCOOH} + \underset{\substack{\| \\ \mathrm{N{-}R''}}}{\overset{\substack{\mathrm{N{-}R'} \\ \|}}{\mathrm{C}}} \xrightarrow{\mathrm{H^+}} \mathrm{RCOO{-}C}(=\overset{+}{\mathrm{N}}\mathrm{H{-}R'})(\mathrm{NH{-}R''}) \xrightarrow{\mathrm{HX}} \mathrm{R}\overset{\overset{\mathrm{O}}{\|}}{\mathrm{C}}\mathrm{X} + \mathrm{H^+} + \mathrm{O{=}C}(\mathrm{NHR'})(\mathrm{NHR''}) \qquad (1)$$

Kinetic studies with simple carboxylic acids led to conditions giving quantitative yields, for example, the reaction of *m*-nitrobenzoic acid (5×10^{-3} *M*), glycine methyl ester (1.0 *M*), and BDC (0.10 *M*). Aliquots were quenched with ten volumes of 1.0 *M* acetate buffer at pH 4.75, acidified with 4 *M* HCl, extracted with chloroform, and the *m*-nitrobenzoic acid back-extracted into carbonate buffer at pH 9.5. The product, methyl *m*-nitrohippurate, was estimated from its absorption at 255 mμ in the chloroform layer, and a graph of yield *vs.* time is shown in Figure 1, with a theoretical first-order curve calculated from $k = 1.5 \times 10^{-3}$ sec^{-1} and an ultimate yield of 100%.

To test this procedure on protein carboxyl groups, the enzymes lysozyme, chymotrypsin, and trypsin (at 10 mg/ml) were allowed to react with 1.0 *M* glycine methyl ester hydrochloride and 0.10 *M* BDC at 25° and pH 4.75 in a pH-Stat. The reaction mixture was quenched by dilution with ten volumes of 1.0 *M* acetate buffer at pH 4.75 and extensively dialyzed. The number of glycine methyl ester residues introduced per mole of enzyme was then calculated from the additional covalently bound

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(1) H. Fraenkel-Conrat and H. S. Olcott, *J. Biol. Chem.*, **161**, 259 (1945).

(2) C. A. Broomfield, J. P. Riehm, and H. A. Scheraga, *Biochemistry*, **4**, 751, 760 (1965).

(3) B. Keil and F. Sorm, *Chem. Listy*, **47**, 245 (1953).

(4) A. C. Chibnall, J. L. Mangan, and M. W. Rees, *Biochem. J.*, **68**, 114 (1958).

(5) G. R. Delpierre and J. S. Fruton, *Proc. Natl. Acad. Sci. U. S.*, **54**, 1161 (1965).

(6) A. L. Grossberg and D. Pressman, *J. Am. Chem. Soc.*, **82**, 5478 (1960).

(7) M. S. Doscher and P. E. Wilcox, *J. Biol. Chem.*, **236**, 1328 (1961).

(8) J. P. Riehm and H. A. Scheraga, *Biochemistry*, **4**, 772 (1965).

(9) J. P. Riehm and H. A. Scheraga, *ibid.*, **5**, 99 (1966).

(10) J. G. Sheehan and J. J. Hlavka, *J. Org. Chem.*, **21**, 43 (1956).

(11) C. Franzblau, P. M. Gallop, and S. Seifter, *Biopolymers*, **1**, 79 (1963).

(12) T. L. Goodfriend, L. Levine, and G. D. Fasman, *Science*, **144**, 1344 (1964).

(13) H. G. Khorana, *Chem. Rev.*, **53**, 145 (1953).

(14) J. C. Sheehan, P. A. Cruikshank, and G. L. Boshart, *J. Org. Chem.*, **26**, 2525 (1961).