be recorded as inactive. However, it would seem that only compounds with activities on the order of at least 20% that of cycloheximide would be suitable as practical repellents.

The average highest concentration gnawed in the graded strip test was of the same order as the effective coating concentration in field tests. Thus, for the compounds given in Table I (7.6 and 12 mg. per sq. cm., respectively), in field tests on coated boxes concentrations of 7.7 mg. per sq. cm. were effective repellents. Cycloheximide itself (0.16 mg. per sq. cm.) showed activity at 0.38 mg. per sq. cm., but was even more effective at higher concentrations (*12*). Although individual rats gnawed concentrations much higher than the averages, in the experimental test, the rat was a highly trained, strongly activated (hungry) animal with a strong lure (peanut) always less than an inch from his nose. On the other hand, the rat under field conditions had the alternative of looking for food elsewhere.

Although it has not been possible to carry out field tests using compounds whose activity was first revealed by this assay method, the graded strip offers a simple, reliable method not only for detecting rat repellent activity as coatings on paperboard, but also for a quantitative comparison with known effective repellents.

Table II. Comparison of Four Repellency Test Methods

Chemical	Graded Strip Cycloheximide Activity, %	Food Repellency Index (2% in Diet)	Barrier Tests, Penetration Time Treated/Control (50 Mg./Sq. In.)	Field Tests, Penetration Time Treated/Control (50 Mg./Sq. In.)
Cycloheximide	100	100 (*3*)		>31 at 30 and 10, 24 at 5, 5.6 at 2.5 (*12*)[a]
Aniline–1,3,5-trinitrobenzene complex	3.0[b] 2.1[c]	91.5 (*3*) 100 (*6*)	1.5 (*6*)	10.4 (*6*)
o-Anisidine–1,3,5-trinitrobenzene complex	0.6[d]	98.6 (*3*) 99.3 (*6*)	2.6 (*6*)	9.5 (*6*)
o-Toluidine–1,3,5-trinitrobenzene complex	1.5[d]	100 (*3*, *6*)	7.3 (*6*)	2.0 (*6*)
1-Naphthylamine–1,3,5-trinitrobenzene complex	0.4[d]	100 (*3*, *6*)	3.5 (*6*)	2.6 (*6*)
Zinc dimethyldithiocarbamate–cyclohexylamine complex (Z.A.C.)	1.2[e] 1.4[c]			9.5 3.0 at 20 (*14*)[a]
Bis (dimethylthiocarbamyl) disulfide (tetramethylthiuramdisulfide)	1.3[b]	73, 90, 96 (*3*)	12 (*2*)	3 to 4 at 20 (*14*)[a]
Bis (diethylthiocarbamyl) disulfide (tetraethylthiuramdisulfide)	2.0[b]	69 (*3*)[f]		
Pentabromophenol	0.3[d]	49 (*3*)		
2-Phenylindole	0.3[d]	85, 100 (*3*)		

[a] Recalculated from days until treated and control boxes were penetrated. Undamaged boxes calculated as if penetrated on last day of test. [b] Determined on 18 rats, 8-hour starvation. [c] Determined on 18 rats, 24-hour starvation. [d] Determined on 6 rats, 8-hour starvation. [e] Determined on 12 rats, 8-hour starvation. [f] Value of 91 given by J. B. DeWitt on rodent repellents. Other data agreed with (*3*).

Acknowledgment

The studies reported were made possible by a research grant from The Dow Chemical Co., Midland, Mich. The help of Larissa Kaseoru, Richard E. Stallman, and James D. Wonder is gratefully acknowledged.

Literature Cited

(1) Bellack, E., DeWitt, J. B., *J. Am. Pharm. Assoc., Sci. Ed.* **38,** 109 (1949).
(2) *Ibid.*, **39,** 197 (1950).
(3) Bellack, E., DeWitt, J. B., Treichler, R., Chem-Biological Co-ordination Center, Natl. Research Council, Washington, D. C., Review **5** (1953).
(4) Bliss, C. I., *J. Am. Pharm. Assoc., Sci. Ed.* **29,** 465 (1940).
(5) Burn, J. H., Finney, O. J., Goodwin, L. G., "Biological Standardization," 2nd ed., pp. 32–48, Oxford University Press, London, 1950.
(6) DeWitt, J. B., Bellack, E., Welch, J. F., *J. Am. Pharm. Assoc., Sci. Ed.* **42,** 695 (1953).
(7) Gillespie, R. J. G., Lucas, C. C., *Nature* **180,** 1292 (1957).
(8) Kaseoru, Larissa, "Barrier Penetration Test Method to Determine Efficacy of Rat Repellent Chemicals," thesis, Drake University, Des Moines, Iowa, 1957.
(9) Munn, N. L., "Handbook of Psychological Research on the Rat," p. 250, Riverside Press, Cambridge, 1950.
(10) Traub, R., DeWitt, J. B., Welch, J. F., Newman, Doris, *J. Am. Pharm. Assoc., Sci. Ed.* **39,** 552 (1950).
(11) Weeks, J. R., *J. Pharmacol. Exptl. Therap.* **119,** 193 (1957).
(12) Welch, J. F., J. AGR. FOOD CHEM. **2,** 142 (1954).
(13) Welch, J. F., DeWitt, J. B., Bellack, E., *Soap Sanit. Chemicals* **26,** No. 5, 147 (1950).
(14) Welch, J. F., Duggan, E. W., *Modern Packaging* **25,** No. 6, 1930 (1952).

Received for review June 16, 1958. Accepted December 1, 1958. Fall meeting, American Society for Pharmacology and Experimental Therapeutics, French Lick, Ind., 1956.

FUMIGANT REACTIONS WITH FOODS

Hydroxyethyl Derivatives in Prunes Fumigated with C^{14}-Ethylene Oxide

H. T. GORDON

Department of Entomology and Parasitology, University of California, Berkeley, Calif.

W. W. THORNBURG and L. N. WERUM

Biological Research Laboratory, California Packing Corp., Emeryville, Calif.

FUMIGATION WITH VOLATILE alkylating agents such as methyl bromide or ethylene oxide is an effective way to protect stored food products from insect and fungus damage. Such chemical treatment usually causes some chemical modification of the food product. Winteringham (*18*) has shown that wheat fumigated with methyl bromide reacts chemically with the fumigant with the formation of water-soluble bromide and methylated products.

Dried fruits (prunes, raisins, apricots, etc.) may require fumigation in some instances, but little is known about the reactive constituents in fruit that are likely to be modified by fumigants. This investigation was designed to explore the effect of fumigating dried prunes with ethylene oxide under controlled conditions in order to provide data on the quantity and identity of these relatively unknown food constituents. The fruit selected for this investigation was the dried plum of the French variety grown in the Santa Clara Valley in California. The major constituents

Dried prunes fumigated with ethylene oxide-C^{14} react with the fumigant to give nonvolatile and relatively nontoxic alkylation products. Over 50% of the total radioactivity is combined as insoluble hydroxyethyl cellulose in the prune skin, 30% as hydroxyethylated sugars in the pulp, and 3% as glycols (mostly diethylene glycol). The remainder has been tentatively identified as hydroxyethylated amino acids and proteins.

of prunes are sugars, and there are many minor constituents—flavonoids, pectin, tannins, protein, and free amino acids. A typical analysis of the type of prune taken for this study would include about 40% glucose, 15% fructose, and 26% water; other carbohydrates, such as sucrose are present in small variable amounts (1 to 6%). Other solids include about 3% protein, 1% free amino acids, 1% pectin, and 0.8% phenolic compounds. The remainder is mostly polysaccharide such as cellulose. Many other dried fruits are chemically very similar to prunes.

The use of ethylene oxide labeled with carbon-14 appeared to be the most promising method for the identification of small amounts of epoxide breakdown or reaction products in large amounts of naturally occurring prune constituents. The most satisfactory preparation of ethylene oxide seemed to be the reaction of 2-bromoethanol-1,2-C^{14} (commercially available) with potassium hydroxide in a special microfumigation apparatus. Fractionation and characterization of the radioactive products could then be effected by paper chromatography and ionophoresis.

Materials, Apparatus, and Methods

2-Bromoethanol-1,2-C^{14}, specific activity 0.53 mc. per mmole (Tracerlab, Inc.). A 10-μl. aliquot (74 μc., 17.7 mg., 140 μmoles) was used. To attain the ratio of fumigant to dried fruit necessary for effective commercial fumigation, only one prune was treated with the radioactive ethylene oxide. Figure 1 (exploded view) illustrates the apparatus used to treat the prune. A dried prune weighing 5.85 grams was placed in the top chamber (volume 16 ml.). Two hundred microliters of potassium hydroxide (60%) were placed in the large compartment of the bottom chamber (volume 0.35 ml.). The C^{14}-ethylene bromohydrin was placed in the other compartment of the bottom chamber (volume 0.10 ml.). The bottom chamber was opened to the top chamber containing the prune, and a partial vacuum was drawn on both chambers. The upper stopcock was closed and the apparatus tipped so that the potassium hydroxide ran into the ethylene bromohydrin. The bottom chamber was dipped in boiling water for 1.5 minutes, then closed off from the top chamber. Radiometric counting of samples from both chambers indicated that 96% of the bromohydrin was converted to ethylene oxide, of which 134 μmoles (5.8 mg., 71 μc.) were therefore synthesized. The prune was allowed to remain in the gas for 6 days. After this time the prune was removed and kept in a freezer. Upon calculating recoveries of the radioactive materials, most of the radioactive ethylene oxide was accounted for as prune addition products, and only traces of ethylene oxide gas were recovered from the fumigation chamber. Absorption and chemical reaction of the ethylene oxide are therefore essentially complete after 6 days in the sealed chamber, even at the fumigant level equal to 0.1% of the weight of the fruit.

One - Dimensional Paper Electrophoresis. The equipment used was a conventional flat plate electrophoresis apparatus (Microchemical Specialties Co., Berkeley, Calif.). The solutions were organic buffers of pH 3.3, 4.7, 7.2, and 9.3, and one borate buffer of pH 9.3; they will be described in detail in a separate publication (*16*).

One-Dimensional Paper Chromatography. Solutions and apparatus for one-dimensional paper chromatography of carbohydrates using solvent IPWA (isopropyl alcohol, pyridine, water, and acetic acid in the volume ratio 8:8:4:1) have been described (*7, 8*). A second solvent, BuPWA (isobutyl alcohol, pyridine, water, and acetic acid in the volume ratio 12:6:4:1) was developed in the course of this work for the separation of compounds having too high R_f values in IPWA.

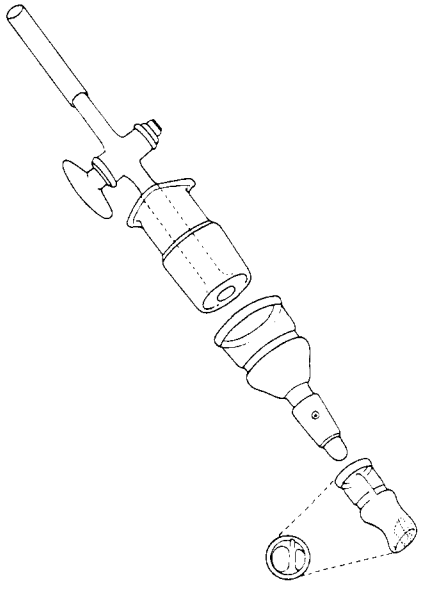

Figure 1. Magnified view of microfumigation apparatus

Hydrolysis. Two methods of hydrolysis were used to degrade the various fractions separated from the prune. In both methods of hydrolysis 3 μl. of ethylene glycol were added as a carrier to the hydrolyzates, before they were concentrated and spotted on chromatograms, in order to lessen the loss of radioactive ethylene glycol by evaporation. The first method was a 6-hour 100° C. hydrolysis with 5 ml. of 0.1*M* aqueous barium hydroxide. Barium ions were removed by the addition of a calculated amount of sulfuric acid, to eliminate salt interference in paper chromatography. Alkaline hydrolysis does not split the alkoxyl groups, and polysaccharides can be degraded to monosaccharides without removing the radioactive 2-hydroxyethyl groups.

The second method (which was used to a lesser extent) was a 12-hour hydrolysis with 2 to 4 ml. of boiling 25% sulfuric acid. Sulfate ions were removed by the addition of a calculated amount of barium hydroxide. Alkoxyl bonds were broken and ethylene glycol was formed proving that ethylene oxide had been bound in an ether linkage.

Nitric Acid Oxidation. Glycols were oxidized to acids for identification by paper chromatography. Three microliters of nonradioactive ethylene glycol were added to an ether extract of prune material, the ether was evaporated, and 0.3 ml. of concentrated nitric acid was added. The mixture was slowly evaporated on a hot plate, but not to dryness. Residual nitric acid was removed by successive addition of 0.3 ml. of water and evaporation (never to dryness, to avoid loss of glycol oxidation products).

Radiometry of Carbon-14–Labeled Prune Material. Quantitative determinations were done by applying coarsely ground powders or aliquots of solutions on planchets, which were then counted. Observed counts were then roughly corrected for losses due to self absorption, geometry, etc., to enable conversion from counts per minute to microcuries.

Paper chromatograms and ionograms were scanned for radioactive spots in a gas flow Geiger tube scanner connected to a rate-meter and pen recorder. Areas of peaks on the record were measured, and found to be roughly proportional to the count obtained by eluting and plancheting the spots. Methods giving highly precise data were not used, because the data would be valid for only one prune, which might not be average in every way. Also, even a 20% error in the values given by the imprecise

methods will not seriously alter the picture of the distribution of ethylene oxide in various prune fractions.

Results

Uptake of Carbon-14 Ethylene Oxide by Different Tissue Fractions of the Prune. The prune was separated mechanically into several tissue fractions, as indicated in Table I. Aliquots were plancheted and counted, and the radioactivity was estimated (using empirical corrections for self-absorption). The pulp was further fractionated into ether-extractable material, water-extractable material, and insoluble residue. Table I shows that the skin has adsorbed the major fraction of the ethylene oxide, and that most of the remainder is in the water-soluble fraction of the pulp.

The Carbon-14–Labeled Constituents of Prune Skin. In this work the prune skin was defined as the covering of the prune which was freed of water-soluble material. This skin is mostly cellulose, but probably contains small amounts of other polysaccharides. From the radioactivity of the skin (35 μc. per gram) and the specific activity of the ethylene oxide used (0.236 mg. per μc.), we can calculate that about 0.8% of the weight of the skin is added radioactive ethylene oxide. The thorough studies of Croon and Lindberg (*4*) on the reaction of ethylene oxide with cellulose have shown that as much as 14% by weight of ethylene oxide can be added to cellulose (when a large excess of ethylene oxide is used). Croon and Lindberg found that most of the added ethylene oxide could be isolated as 6-(2-hydroxyethyl)glucose, 6-(2-hydroxyethoxyethyl)glucose, 2-(2-hydroxyethyl) glucose, and 2-(2-hydroxyethoxyethyl)glucose. Small amounts of many other substituted glucoses were found.

Chromatography of a barium hydroxide hydrolyzate of prune skin in solvent IPWA gave one broad spot at R_f 0.78, indicating a mixture of similar compounds. Ionophoresis of another portion of the hydrolyzate at pH 9.3 in dimethylaminoethanol acetate buffer gave only one spot of zero mobility, but ionophoresis in borate buffer at pH 9.3 gave four spots. Table II compares the M_G values of these spots with values for known alkylated glucoses. The M_G is the ionic mobility of a substance relative to that of glucose, which is taken as 1.0. Table II shows the excellent correlation between the ethylene oxide–treated prune skin and the ethylene oxide–treated cellulose of Croon and Lindberg. The major products from prune skin are certainly 6-(2-hydroxyethyl) glucose (spot *A*) and 6-(2-hydroxyethoxyethyl)glucose (spot *B*). The high radioactivity at M_G zero (spot *D*) can be accounted for by incomplete hydrolysis products of the cellulose (cellobioses and other oligosaccharides), by degradation products of the (2-hydroxyethyl)glucoses to furfural derivatives, and by a residue of tannins and other minor constituents of prune skin, which is not pure cellulose. Each spot obtained in the course of this work (and usually not further fractionated) is given an identifying letter (*A*, *B*, etc.). If two spots from different sources are believed to represent identical substances, they are given the same letter.

Table I. Radioactivity in Fractions of C^{14}-Ethylene Oxide–Treated Prune

Prune Tissue	Total Weight, Mg.	Observed, Counts/ Min./ Mg.	Estimated[a] Millimicrocuries/ Mg.	Approximate Total, Microcuries	Total Activity, %
Skin	1290	3370	35	45	63
Pulp, whole[b]	3750	1160	7	25	
Ether-extractable	8	40500	48	0.4	0.5
Water-extractable	2550	1510	9	22	31
Insoluble residue	190	3020	18	3	4
Pit (less kernel)	650	119	1.4	0.8	1
Kernel	162	54	0.6	0.1	0.1

[a] Empirically determined factors ranging from 0.0012 (for samples with negligible self-absorption) to 0.014 (for samples with very high self absorption) were used to convert counts/minute to millimicrocuries. Activity of original C^{14}-ethylene oxide is 4200 mμc./mg., and would be 3000 mμc./mg. if converted to ethylene glycol.
[b] Whole pulp weight includes 26% water, not included in weights of 3 subfractions below.

Table II. Tentative Identification of Constituents of Alkaline Hydrolyzate of Radioactive Prune Skin

Prune Skin Hydrolyzate			Known Compounds[a]		
Spot	% of total radioactivity	M_G	M_G	Ethylene oxide[a] expected, %	Name
A	33	0.81	0.83	33	6-(2-Hydroxyethyl)glucose
			0.71	3	3-(2-Hydroxyethyl)glucose
B	21	0.67	0.67	22	6-(2-Hydroxyethoxyethyl)glucose
C	~5–15	~0.46 (broad)	0.63	2	3-(2-Hydroxyethoxyethyl)glucose
			0.52	2	3,6-Di(2-hydroxyethyl)glucose
			0.32	10	2-(2-Hydroxyethyl)glucose
			0.24	7	2-(2-Hydroxyethoxyethyl)glucose
D	~30–40	~0 (broad)	0.22	5	2,6-Di(2-hydroxyethyl)glucose
			(0?)	15	Mixture of isomeric (2-hydroxyethyl)cellobioses
			(0?)	(15)	Mixture of degradation products of all of the above compounds, and also of (2-hydroxyethyl)pentosan and tannins

[a] All data for known compounds are from Table I of Croon and Lindberg (*4*), and agree with data for similarly methyl-substituted glucoses (*5*). Values for % distribution of total ethylene oxide into each compound are not given by Croon and Lindberg, but were calculated from their quantitative data.

The Carbon-14–Labeled Ether-Extractable Constituents of Prune Pulp. When ethylene oxide reacts with water, ethylene glycol and polyethylene glycols are formed. Ether will completely extract these glycols from prune pulp. To minimize evaporative losses, 2 to 5 μmoles of nonradioactive ethylene glycol were added to ether extracts before evaporation and chromatography.

Paper chromatography of the ether-soluble fraction gave one broad spot at R_f 0.87 in solvent IPWA and R_f 0.72 in BuPWA. Heating the chromatogram for 30 minutes at 110° C. caused a 40% loss in radioactivity of the spot, proving that much of the activity is in a somewhat volatile compound. For qualitative identification of alcohols by paper chromatography, the standard technique is to prepare derivatives such as the potassium alkylxanthates or the half esters with 3-nitrophthalic acid (*12*). With dihydric alcohols, however, it is simpler to oxidize to dicarboxylic acids with nitric acid as described under Methods. Nitric acid oxidation of the ether-extractable fraction gave three new spots on the chromatograms, with small and variable spots of the unoxidized material. Table III compares these spots with those of known oxidation products of glycols. The major constituent of the ether extract seems to be diethylene glycol.

The dried prune contains 26% water, but much of this is not wholly "free," and forms a viscous hydrated sirup with large amounts of hexose sugars. Ethylene oxide will react more readily with a molecule of ethylene glycol (already formed by reaction with water) than with another molecule of water to form more ethylene glycol. Diethylene glycol (and probably some triethylene glycol) should therefore be the major product of this reaction sequence. Nitric acid

Table III. Tentative Identification of Ether-Soluble Radioactive Constituents of Prune Pulp (Oxidized with Nitric Acid)

Radioactive Spots			Known Substances		
Spot	R_f in IPWA	R_f in BuPWA[a]	R_f in IPWA	R_f in BuPWA	Name
E	0.35	0.10	0.33	0.12	Oxalic acid
F	0.62	0.25	0.55	0.23	Diglycolic acid
			0.74	0.45	Glycolic acid
G	0.66	0.59	0.70	0.55	2-Hydroxyethylglycolic acid[a]
H	0.87	0.72	0.87	0.74	Ethylene glycol[a] Diethylene glycol[a] Triethylene glycol[a]

[a] Heating of paper strip at 110° C. for 30 minutes causes a 70% loss of radioactivity in spot *G*, slight loss of activity in spot *H*, and no loss in spots *E* and *F*. Spots of nonradioactive 2-hydroxyethylglycolic acid are relatively volatile, because of formation of cyclic lactone. Major fraction of radioactivity in all runs was found in spot *G* or *F*.

Table IV. Tentative Identification of Water-Soluble Radioactive Constituents of Prune Pulp[a]

Prune Pulp Constituents			Known Compounds		
Spot	% of total radioactivity	M_G	M_G	Name	Reference
A	64	0.81	0.83	6-(2-Hydroxyethyl)glucose	(*4*)
J	8	0.46	? 0.32	1- or 6-(2-hydroxyethyl)fructose 2-(2-Hydroxyethyl)glucose	None (*4*)
K	28	0.17	? 0.17 ? 0.17	1-(2-Hydroxyethyl)glucose 1-Ethylglucose *x*-(2-hydroxyethyl)sucrose Sucrose	None (*6*) None (*6*)

[a] Ionophoretic subfractionation of fraction *Y*, isolated from pulp water-solubles by preparative scale paper chromatography, contains 80% of the water-soluble radioactivity.

seems to oxidize ethylene glycol much more quickly than diethylene glycol, and only oxalic acid (spot *E*) is formed (the intermediate, glycolic acid, being very easily oxidized). Diethylene glycol remains partly unoxidized (spot *H*), and is slowly oxidized to the monocarboxylic acid (spot *G*), which is slowly oxidized to the dicarboxylic acid (spot *F*). Some cleavage of the ether linkage (and rapid oxidation to oxalic acid) may also occur.

Carbon-14–Labeled Water-Soluble Constituents of Prune Pulp. Paper chromatography of the water extract of prune pulp gave three spots: spot *X* (about 13% of the radioactivity) having R_f zero in both IPWA and BuPWA, spot *Y* (about 79% of the activity) being a broad spot with R_f 0.75 in IPWA and 0.45 in BuPWA, and spot *Z* (about 8% of the activity), poorly resolved from spot *Y* in IPWA, but forming a broad band from R_f 0.55 to 0.85 in BuPWA.

It seemed possible that the major spot was a mixture of 2-hydroxyethyl–substituted sugars, because 60 to 70% of the weight of prune pulp is water-soluble sugars. The R_f value of the main constituent (glucose) is 0.65 in IPWA and 0.29 in BuPWA, but the addition of a 2-hydroxyethyl group will raise the R_f value. To isolate the small amount of radioactive sugar from the bulk of the inert sugar, preparative scale chromatography (using 9 × 11 inch sheets of Whatman No. 4 paper and the BuPWA solvent) was necessary. The radioactive band *Y* (R_f 0.35 to 0.55) and band *X* (R_f 0) were cut out for further study. Band *Z* was not investigated, because it seems to be a complex mixture of many substances, each comprising only a minute fraction of the total radioactivity. It is probably mostly *N*-2-hydroxyethyl–substituted amino acids. This fraction may constitute less than 1% of the weight of prune pulp, and its high radioactivity indicates a high ethylene oxide addition (possibly 50 to 60 μmoles per gram).

Ionophoresis of the "radioactive sugar" fraction *Y* at pH 3.3 and at pH 9.3 showed that all the radioactive material in this fraction had zero ionic mobility. In borate buffer at pH 9.3, however, all the radioactive material moved. Table IV gives M_G values and tentative identification of three major spots.

The fact that fraction *X* of the water-solubles in prune pulp has R_f zero in IPWA (with no indication of upward streaking) demonstrates strongly that it consists of relatively polar molecules linked together into a macromolecule of fairly high molecular weight. Prunes contain three major types of high-polymer water-soluble substances: proteins, pectins, and tannins. Fraction *X* must be more strongly labeled than the sugars of fraction *Y*, because it contains 13% of the radioactivity, but cannot constitute more than 3 to 6% of the weight (content of ethylene oxide about 20 to 40 μmoles per gram). The polymer must therefore contain many groups that react very readily with ethylene oxide (such as imidazole, amino, sulfhydryl, or thioether groups) in order to absorb twice as much per unit weight. The side chains of lysine, histidine, cysteine, and methionine in wheat protein are heavily methylated in wheat fumigated with methyl bromide (*18*), and it is probable that hydroxyethylation of fraction *X* is likewise largely in the side chains of histidine and methionine of the prune protein. Acid or alkaline hydrolysis gives small amounts of many compounds whose R_f values (0.22, 0.27, 0.48, 0.59, and 0.74 in BuPWA) are compatible with the expected values for hydroxyethylamino acids.

Ionophoresis of the total water-soluble extract (containing fractions *X*, *Y*, and *Z*) in buffer at pH 9.3 showed that about 85 to 90% of the radioactivity (mostly fraction *Y*) had zero ionic mobility, but that the remainder (10 to 15%, mostly fractions *X* and *Z*), was a mixture of negatively charged compounds varying in mobility, but within the range of known amino acids and proteins.

Carbon-14–Labeled Water-Insoluble Constituents of Prune Pulp. The small amount of fibrous material (about 5% of total weight) which remained after ether and water extraction of prune pulp was partly cellulose, but its radioactivity was surprisingly high (about 12% of the total activity in the pulp). Paper chromatography of a barium hydroxide hydrolyzate gave one broad spot in each solvent (R_f 0.7 in IPWA and 0.4 in BuPWA). Ionophoresis in borate buffer at pH 9.3 gave two spots, the major one (64% of the radioactivity) at M_G 0.81 being probably spot *A*, 6-(2-hydroxyethyl)glucose. The minor spot (spot *D*, 30% of the activity) is broad and has a peak at M_G 0.06, and is probably mostly (2-hydroxyethyl)cellobiose. This is unlike the pattern obtained from an alkaline hydrolyzate of prune skin, and forces the conclusion that the pulp cellulose, unlike the skin cellulose, is only lightly labeled with ethylene oxide. If only the reactive hydroxyls at C-6 are labeled, the addition of ethylene oxide should be of the same order of magnitude as that on the free glucose (about 10 μmoles per gram). The other component of the pulp water-insoluble fraction must therefore be strongly labeled with ethylene oxide (30 to 40 μmoles per gram, if it constitutes half of the weight of this fraction). By analogy with fraction *X* of the pulp water-solubles, we may speculate that the heavily labeled material in the pulp water-insolubles is also protein. Much of this probably is degraded to insoluble humins during hydrolysis of the mixture of cellulose

Table V. Distribution of Hydroxyethyl Derivatives in Ethylene Oxide-Fumigated Prunes

Solubility of Derivative	Prune Constituent Labeled[a]	Location in Prune	Total Radioactivity, %	Major 2-Hydroxyethyl H Derivatives	Reactive Groups Labeled, %
Insoluble	...	Pit	1		
	Cellulose	Skin	63	6-H- and 6-HH-cellulose	~13
	Cellulose	Pulp	(1)	6-H-cellulose	~ 1
	(Protein)	Pulp	(3)	(H-histidine and H-methionine)	~15
Water-soluble	(Protein)	Pulp	(4)	(H-histidine and H-methionine)	
	(Amino acids)	Pulp	(3)	(H-amino acids)	~ 5–15
	Sugars	Pulp	24	6-H-glucose and 1-H-glucose	0.7
Ether-soluble	Water	Pulp	0.4	Diethylene glycol, ethylene glycol	0.0003

[a] Names in parentheses indicate tentative or speculative identification only.

and proteins, but some of it may be present in spot *D* of the borate ionophoresis, together with incompletely hydrolyzed products from the cellulose.

Summary and Conclusions

Table V summarizes the results of this work, which have shown conclusively that over 50% of the ethylene oxide has added to cellulose (nearly all in the prune skin) and over 20% to free sugars (mostly glucose), the dominant labeling being on the primary alcohol group (C-6) of the glucose units. Fractions tentatively identified as protein and free amino acids are two to six times more active per unit weight, but represent only 10% of the total ethylene oxide addition. Some of the material listed in Table V as amino acids or proteins may in fact be pectin, tannin, or minor constituents such as phenolic glycosides, but these probably contain no more than 2 to 3% of the total ethylene oxide added to the prune. Only 0.5% of the added oxide reacted with water.

These results are different from those of Winteringham (*18*, *19*), who studied the reaction of C^{14}-methyl bromide on wheat flour. Methyl bromide is much less reactive than ethylene oxide, because only 10 to 20% is bound by wheat in a 4-day fumigation (using a dosage of about 20 μmoles per gram of wheat), while 50 to 75% of an equivalent fumigation with ethylene oxide is bound in 4 days (*13*). The higher uptake of ethylene oxide must be due to the fact that, unlike methyl bromide, it reacts strongly with the hydroxyl groups in starch (*11*). Winteringham found that wheat protein was very strongly methylated (about 180 μmoles per gram), and water also reacted strongly with methyl bromide (20 μmole per gram), while starch reacted weakly (3 μmoles per gram). Methylation of the protein side chains was largely on the histidine imidazole groups (37%) and the methionine sulfur (31%). It is probable that much of the ethylene oxide tentatively assigned to prune protein in Table V is similarly bound (*17*). Such alkyl derivatives of amino acids are relatively nontoxic (*14*, *18*), and represent a negligible nutritive loss.

The major fraction of the ethylene oxide is bound to prune cellulose. Cellulose is poorly digestible (*3*), and hydroxyethylcellulose should be even less so, so that neither loss of nutritive value nor formation of absorbable toxic compounds need be considered. Because prune pits are inedible, one can similarly ignore the possible nutritional or toxicological significance of the hydroxyethyl compounds formed in very small amount in the pit and kernel.

The hydroxyethylated sugars in prune pulp, however, are present in fairly large amount (0.5 to 1% of the dry weight). Little is known of the toxicity of alkylated sugars, although 3-methylglucose has been shown to be completely nontoxic to the rat (*2*). Fumigation of complete rat diets (including carbohydrates) with ethylene oxide neither forms toxic compounds nor destroys the nutritive value (*15*), although very high levels of ethylene oxide can inactivate much of the thiamin (*10*) and some histidine and methionine (*17*).

The only known toxic products likely to be formed from ethylene oxide are ethylene glycol and diethylene glycol. The quantity in fumigated prunes would be minute (0.002% of the dry weight), but use of other fumigants (such as propylene oxide or β-propiolactone) would be even less objectionable. Beta-propiolactone has been used at enormous dosages (2 to 3 grams per liter) to inactivate viruses in human plasma, and injection of large volumes of the treated plasma into human beings caused no adverse effects (*9*).

It therefore seems possible to extend Winteringham's conclusion that "there is no evidence of risk to consumers' health as a result of using methyl bromide as a wheat or flour fumigant" to include some other alkylating agents and foods such as dried fruits. In fact, except for the peculiar reaction of nitrogen trichloride on wheat methionine to form methionine sulfoximine (*1*), fumigants apparently form relatively stable and inert derivatives, which cause no greater alteration of food than that resulting from cooking or the normal deterioration on aging, and much less than that resulting from even minor spoilage by bacteria, fungi, or insects. Effective fumigation does not render food permanently immune to spoilage, by converting the bulk of the food constituents into nonnutritious derivatives. It inactivates a relatively small fraction of some highly reactive groups (such as sulfhydryl groups), and this suffices to kill bacterial or fungus spores already present. Introduction of new spores, however, will lead to immediate spoilage, because fumigation has not decreased the nutritive value of the food, nor added to the food any permanent protectant (such as the calcium propionate widely used in bread). The effect is quantitatively similar to heat sterilization.

Literature Cited

(1) Campbell, P. N., Work, T. S., Mellanby, E., *Biochem. J.* **48,** 106 (1951).
(2) Campbell, P. N., Young, F. G., *Ibid.*, **52,** 439 (1952).
(3) Conrad, H. E., Watts, W. R., Iacono, J. M., Kraybill, H. F., Friedemann, T. E., *Science* **127,** 1293 (1958).
(4) Croon, Ingemar, Lindberg, Bengt, *Svensk Papperstid.* **59,** 794 (1956).
(5) Foster, A. B., *J. Chem. Soc.* **1953,** 982.
(6) Foster, A. B., Stacey, M., *J. Chem. Soc.* **1955,** 1778.
(7) Gordon, H. T., Hewel, C. A., *Anal. Chem.* **27,** 1471 (1955).
(8) Gordon, H. T., Thornburg, W. W., Werum, L. N., *Ibid.*, **28,** 849 (1956).
(9) Hartmann, F. W., Kelly, A. R., LoGrippo, G. A., *Gastroenterology* **28,** 244 (1955).
(10) Hawk, E. A., Mickelsen, O., *Nutrition Revs.* **14,** 55 (1956).
(11) Kerr, R. W., U. S. Patent **2,732,309** (1956).
(12) Lederer, E., Lederer, M., "Chromatography," 2nd ed., pp. 157–8, Elsevier, New York, 1957.
(13) Lubatti, O. F., Harrison, A., *J. Soc. Chem. Ind.* **63,** 353 (1944).
(14) Meister, A., "Biochemistry of the Amino Acids," Academic Press, New York, 1957.
(15) Oser, B. L., *Food Technol.* **10,** 175 (1956).
(16) Werum, L. N., Gordon, H. T., Thornburg, W. W., manuscript in preparation.
(17) Windmueller, H. G., Engel, R. W., *Federation Proc.* **15,** 386 (1956).
(18) Winteringham, F. P. W., *J. Sci. Food Agr.* **5,** 269 (1955).
(19) Winteringham, F. P. W., Harrison, A., Bridges, R. G., Bridges, P. M., *Ibid.*, **5,** 251 (1955).

Received for review October 3, 1958. Accepted January 12, 1959.