JOC Note

Substituent Effects upon the Catalytic Activity of Aromatic Cyclic Seleninate Esters and Spirodioxyselenuranes That Act as Glutathione Peroxidase Mimetics

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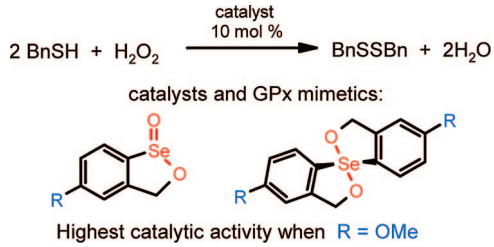
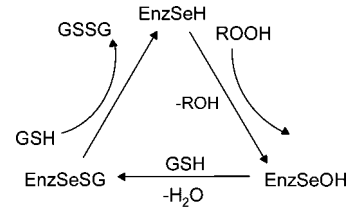


Substituent effects were studied in a series of aromatic cyclic seleninate esters and spirodioxyselenuranes that function as mimetics of the antioxidant selenoenzyme glutathione peroxidase. The methoxy-substituted selenurane proved the most efficacious catalyst for the reduction of hydrogen peroxide with benzyl thiol, and the reaction rates were enhanced for both classes by electron-donating substituents. Hammett plots indicated $\rho = -0.45$ and -3.1 for the seleninates and selenuranes, respectively, suggesting that oxidation at Se is the rate-determining step in their catalytic cycles.

Aerobic metabolism produces peroxides, other reduced oxygen species, and free radicals that contribute to oxidative stress in living organisms. This has been implicated in a variety of degenerative processes and disease states in human patients, including inflammation, cardiovascular disease, mutagenesis and cancer, dementia, and possibly even aging.[1,2] These deleterious effects are mitigated by a variety of dietary antioxidants,[1b,3,4] as well as by several endogenous enzymes that catalyze the destruction of peroxides and other harmful reduced oxygen species. The selenoenzyme glutathione peroxidase (GPx) performs a vital role in this regard by catalyzing the reduction of peroxides with glutathione (GSH), a tripeptide thiol that is abundant in the cells of higher organisms and serves as a stoichiometric reductant in this process. The catalytic cycle of the enzyme has been established and is shown in Scheme 1.[5] The selenol moiety (EnzSeH) associated with each selenocysteine residue in the enzyme[6] reduces peroxides readily and is itself oxidized to the corresponding selenenic acid (EnzSeOH). The latter then reacts with two molecules of GSH to regenerate the selenol via the selenenyl sulfide EnzSeSG, along with a stoichiometric amount of glutathione disulfide (GSSG). At high peroxide concentrations, the corresponding seleninic acid intermediate ($EnzSeO_2H$) may also play a role in the process.

SCHEME 1



EnzSeH = glutathione peroxidase
GSH = glutathione

The design, synthesis, and evaluation for GPx-like activity of small-molecule selenium compounds, as well as of selenium-containing macromolecules, have been the subject of considerable investigation.[7] Ebselen (**1**)[8,9] and ALT 2074 (**2**)[10] are undergoing clinical trials as antioxidants, particularly for counteracting high levels of oxidative stress associated with strokes and related cardiovascular conditions. We recently discovered two types of compounds that function as exceptionally effective GPx mimetics. Thus, the novel cyclic seleninate ester **3**[11] and spirodioxyselenurane **4**,[12] as well as derivatives

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(2) (a) *Free Radicals in Biology*; Pryor, W. A., Ed.; Academic Press: New York, 1976−1982; Vols. 1−5. (b) *Free Radicals in Molecular Biology, Aging and Disease*; Armstrong, D., Sohal, R. S., Cutler, R. G., Slater, T. F., Eds.; Raven Press: New York, 1984.

(3) *Oxidative Processes and Antioxidants*; Paoletti, R., Samuelsson, B., Catapano, A. L., Poli, A., Rinetti, M., Eds.; Raven Press: New York, 1994.

(4) For this reason, selenium is an essential nutrient; see: (a) *Selenium in Biology and Human Health*; Burk, R. F., Ed.; Springer-Verlag: New York, 1994. (b) Shamberger, R. J. *Biochemistry of Selenium*; Plenum Press: New York, 1983.

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(6) The structure of GPx is tetrameric, with a selenocysteine residue in each of the four subunits: Epp, O.; Ladenstein, R.; Wendel, A *Eur. J. Biochem.* **1983**, *133*, 51.

(7) For reviews, see: (a) Mugesh, G.; du Mont, W.-W.; Sies, H. *Chem. Rev.* **2001**, *101*, 2125. (b) Mugesh, G.; Singh, H. B. *Chem. Soc. Rev.* **2000**, *29*, 347. (c) Mugesh, G.; du Mont, W.-W. *Chem. Eur. J.* **2001**, *7*, 1365.

(8) (a) Müller, A.; Cadenas, E.; Graf, P.; Sies, H. *Biochem. Pharmacol.* **1984**, *33*, 3235. (b) Wendel, A.; Fausel, M.; Safayhi, H.; Tiegs, G.; Otter, R. *Biochem. Pharmacol.* **1984**, *33*, 3241. (c) Parnham, M. J.; Kindt, S. *Biochem. Pharmacol.* **1984**, *33*, 3247. (d) Müller, A.; Gabriel, H.; Sies, H. *Biochem. Pharmacol.* **1985**, *34*, 1185. (e) Safayhi, H.; Tiegs, G.; Wendel, A. *Biochem. Pharmacol.* **1985**, *34*, 2691. (f) Wendel, A.; Tiegs, G. *Biochem. Pharmacol.* **1986**, *35*, 2115. (g) Fischer, H.; Dereu, N. *Bull. Soc. Chim. Belg.* **1987**, *96*, 757. (h) Haenen, G. R. M. M.; De Rooij, B. M.; Vermeulen, N. P. E.; Bast, A. *Mol. Pharmacol.* **1990**, *37*, 412. (i) Glass, R. S.; Farooqui, F.; Sabahi, M.; Ehler, K. W. *J. Org. Chem.* **1989**, *54*, 1092.

(9) For a list of potential medicinal applications of ebselen, see: Fong, M. C.; Schiesser, C. H. *Tetrahedron Lett.* **1995**, *36*, 7329.

(10) ALT 2074 was formerly known as BXT 51072. For lead references, see: (a) Moutet, M.; D'Alessio, P.; Malette, P.; Devaux, V.; Chaudière, J. *Free Radical Biol. Med.* **1998**, *25*, 270. (b) Erdelmeier, I.; Tailhan-Lomont, C.; Yadan, J.-C. *J. Org. Chem.* **2000**, *65*, 8152. (c) Jacquemin, P. V.; Christiaens, L. E.; Renson, M. J.; Evers, M. J.; Dereu, N. *Tetrahedron Lett.* **1992**, *33*, 3863.

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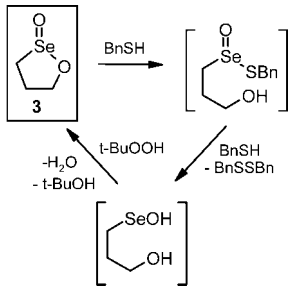
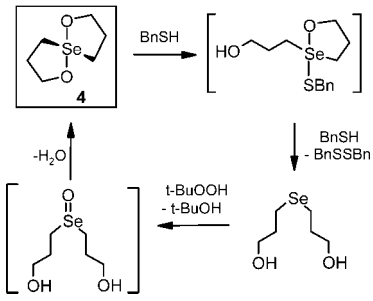
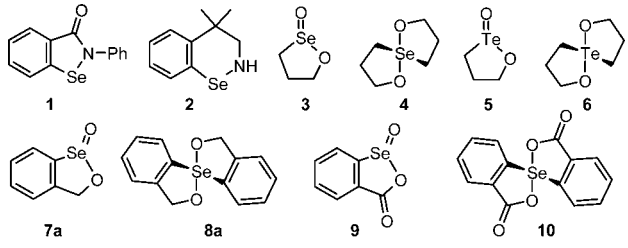
SCHEME 2



SCHEME 3

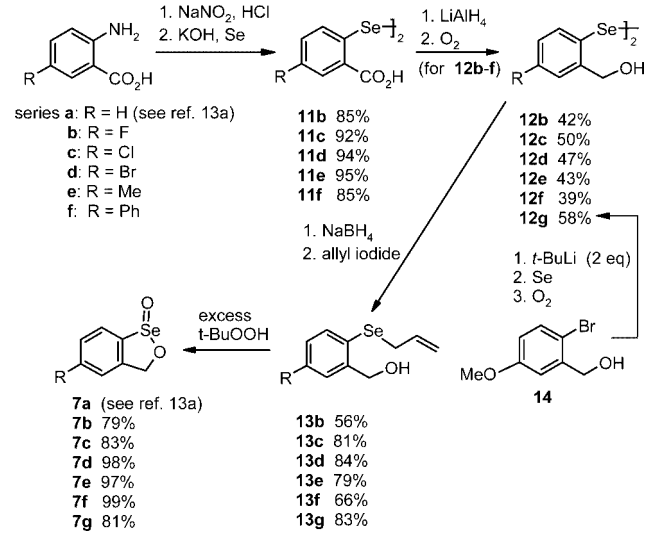


of these prototypes,[13] catalyzed the reduction of *tert*-butyl hydroperoxide or hydrogen peroxide, using benzyl thiol as the sacrificial thiol. The catalytic cycles, shown in Schemes 2 and 3, respectively, proved distinct from each other and from that of GPx, as shown in Scheme 1. Moreover, selenol and selenolate intermediates are capable of reducing dioxygen, resulting in the formation, as well as the destruction, of reactive oxygen species.[14] The absence of selenol intermediates from Schemes 2 and 3 is therefore a further advantage. We also reported that **5** and **6**, the tellurium analogues of **3** and **4**, respectively, displayed greater catalytic activity, while the benzo derivatives **7a**, **8a**, **9**, **10** were considerably less active.[13a] Compounds **7a** and **8a** were also investigated by Singh and co-workers.[15]



In order to compare the relative catalytic activities of various GPx mimetics, we devised a simple assay[11,12,16] in which the catalyst (10 mol %), benzyl thiol (BnSH), and an excess of either *tert*-butyl hydroperoxide or hydrogen peroxide are allowed to react at 18 °C in dichloromethane−methanol solution. The reactions are conveniently monitored by HPLC for the disappearance of the thiol or the formation of the corresponding disulfide (BnSSBn). The distinct methylene signals of the benzylic compounds provide an additional means for monitoring the reactions in deuterated solvents by NMR and for investigating intermediates. For convenience, we compared the activities of the catalysts by means of half-lives ($t_{1/2}$), representing the time required for the conversion of 50% of the thiol to its disulfide under the above conditions.

SCHEME 4



In general, aromatic selenium compounds tend to have lower toxicities than their aliphatic counterparts, mainly because of their greater resistance to metabolic degradation to highly toxic inorganic selenium compounds.[7b,17] Unfortunately, the aromatic derivatives **7a**, **8a**, **9**, and **10** displayed debilitated activity relative to the aliphatic analogues **3** and **4**. We now report the effect of substituents on the aromatic compounds **7a** and **8a** as part of an effort to improve their catalytic activities while retaining the expected lower toxicity. Furthermore, these experiments provided additional insight into the mechanism of the respective catalytic cycles of cyclic seleninate esters and spirodioxyselenuranes.

Cyclic seleninate ester **7a** and spirodioxyselenurane **8a** were obtained as described previously.[13a] The desired substituted derivatives **7b**−**f** and **8b**−**e** were prepared from the corresponding diselenides **11b**−**f**, as shown in Schemes 4 and 5. The latter were in turn obtained from 5-substituted anthranilic acids by the same general procedure that was previously employed for the unsubstituted diselenide **11a**.[13a,18] The diselenides were treated with lithium aluminum hydride to reduce their carboxylic acid substituents to benzylic alcohols and their diselenide moieties to selenolates. Direct treatment of the latter with allyl iodide afforded the allyl selenides **13b**−**f** in poor yields. A more efficient procedure involved the air oxidation of the selenolates produced by the lithium aluminum hydride reduction, followed by isolation of diselenides **12b**−**f**. Reduction with sodium borohydride followed by allylation provided improved yields of **13b**−**f**, which were then converted in one step to the products **7b**−**f** by oxidation with excess *tert*-butyl hydroperoxide (Scheme 4).[19] Diselenide **12g** was best prepared by metalation of bromobenzyl alcohol **14**, followed by selenation of the dianion and air oxidation. It was then converted to **7g** in the usual

(11) (a) Back, T. G.; Moussa, Z. *J. Am. Chem. Soc.* **2002**, *124*, 12104. (b) Back, T. G.; Moussa, Z. *J. Am. Chem. Soc.* **2003**, *125*, 13455.
(12) Back, T. G.; Moussa, Z.; Parvez, M. *Angew. Chem., Int. Ed.* **2004**, *43*, 1268.
(13) (a) Back, T. G.; Kuzma, D.; Parvez, M. *J. Org. Chem.* **2005**, *70*, 9230. (b) Kuzma, D.; Parvez, M.; Back, T. G. *Org. Biomol. Chem.* **2007**, *5*, 3213. (c) Back, T. G.; Kuzma, D.; Berkowitz, N. PCT Int. Appl. WO 2007044641, 2007.
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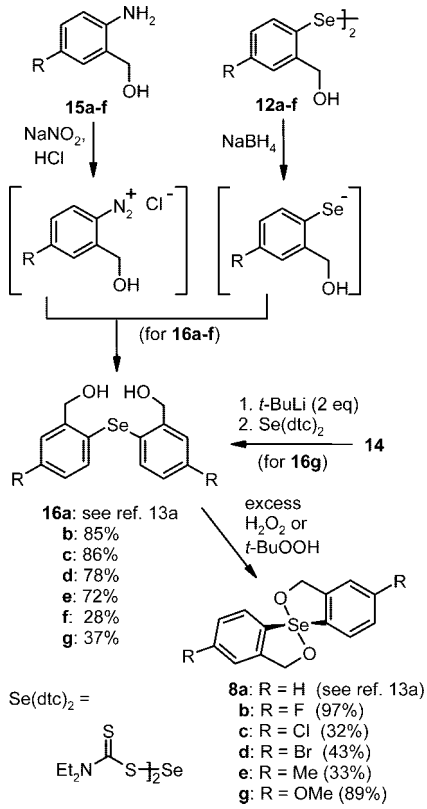
(16) Back, T. G.; Dyck, B. P. *J. Am. Chem. Soc.* **1997**, *119*, 2079.
(17) Nogueira, C. W.; Zeni, G.; Rocha, J. B. T. *Chem. Rev.* **2004**, *104*, 6255.
(18) Lesser, R.; Weiss, R. *Ber. Dtsch. Chem. Ges.* **1913**, 2640.

SCHEME 5

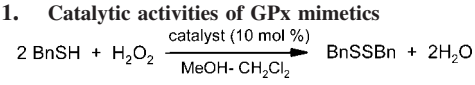


manner. Direct oxidation of diselenides **12b**−**f** with excess hydrogen peroxide or *tert*-butyl hydroperoxide also afforded cyclic seleninates **7b**−**f**, but the products contained impurities which proved difficult to remove.

Diselenides **12a**−**f** were converted to spirodioxyselenuranes **8a**−**f** by reduction to their selenolates, followed by treatment with the diazonium salts generated in situ from 2-aminobenzyl alcohols **15a**−**f**, which were in turn obtained from the reduction of the corresponding anthranilic acids with lithium aluminum hydride. The resulting selenides **16a**−**f** contained small amounts of the corresponding diselenides and were typically oxidized with excess hydrogen peroxide or *tert*-butyl hydroperoxide without further purification to afford the desired spiro compounds. Selenide **16g** was best obtained by selenation of the dianion of **14** with $Se(dtc)_2$[20] (Scheme 5).

Substituted cyclic seleninates **7a**−**g** and spirodioxyselenuranes **8a**−**e**,**g** were then subjected to our assay for catalytic activity, using benzyl thiol and hydrogen peroxide. The phenyl-substituted spirodioxyselenurane **8f** could not be purified completely and proved too insoluble for measurement under the conditions of the assay. The $t_{1/2}$ values thus obtained are indicated in Table 1, along with those of the aliphatic analogues **3** and **4** for comparison. A control reaction performed in the absence of a selenium-based catalyst under the same conditions produced a $t_{1/2}$ of 176 h. These results indicate that the aromatic cyclic seleninate esters **7a**−**g** (entries 3−9) are all inferior to ebselen (**1**; entry 1) and the aliphatic derivative **3** (entry 2) in catalytic activity, as measured by this assay. On the other hand, spirodioxyselenuranes **8a**, **8e**, and **8g** (entries 11, 15 and 17,

TABLE 1. Catalytic activities of GPx mimetics



entry	catalyst	$t_{1/2}$ (h)
1	ebselen (**1**)	24
2	**3**	18[a]
3	**7a**	50
4	**7b**	72
5	**7c**	69
6	**7d**	62
7	**7e**	48
8	**7f**	57
9	**7g**	38
10	**4**	0.2[a]
11	**8a**	2.9
12	**8b**	22
13	**8c**	25
14	**8d**	31
15	**8e**	3.6
16	**8f**	
17	**8g**	0.5

[a] Data taken from ref 13a using slightly different conditions.

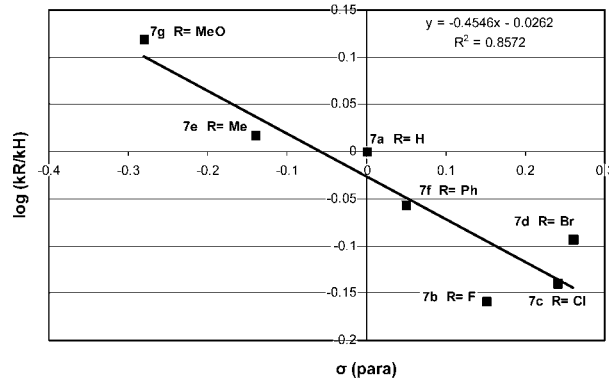


FIGURE 1. Hammett plot for cyclic seleninates **7a**−**g**.

respectively) proved superior to **1**, with the methoxy derivative **8g** (entry 17) displaying a $t_{1/2}$ 48 times smaller than that of **1**, and approaching that of the aliphatic spirodioxyselenurane **4** (entry 10).

Moreover, there appears to be a clear correlation between catalytic activity as measured by the $t_{1/2}$ values in Table 1 and the electron-donating/withdrawing nature of the substituents in both the cyclic seleninates and spirodioxyselenuranes. Thus, while electron-withdrawing substituents such as the halogens in **7b**−**d** and **8b**−**d** (entries 4−6 and 12−14) suppressed catalytic activity, the strongly electron-donating methoxy groups in **7g** and **8g** enhanced it considerably (entries 9 and 17). The effects of the phenyl and methyl substituents in derivatives **7e**, **7f**, and **8e** were relatively modest (entries 7, 8, and 15). The correlation of catalytic activity with substituent effects was also evident in the Hammett plots for the above reactions. Thus, the overall rates for the reactions of benzyl thiol with hydrogen peroxide were measured in the presence of the unsubstituted cyclic seleninate **7a** and the substituted derivatives **7b**−**g**. The Hammett plot was made using conventional values for σ_{para},[21] resulting in a reaction constant $\rho = -0.45$ (Figure 1). The negative slope indicates that the transition state of the rate-determining step is stabilized by electron-donating substituents and destabilized by electron-withdrawing groups. The similar

(19) This process presumably proceeds via selenoxide formation from **13**, [2,3]sigmatropic rearrangement to the corresponding selenenate ester, followed by further oxidation and cyclization to afford **7**.

(20) Foss, O. *Inorg. Synth.* **1953**, *4*, 91.

(21) Smith, M. B.; March, J. *March's Advanced Organic Chemistry*, 5th ed.: Wiley: New York, 2001; p 370 and references cited therein.

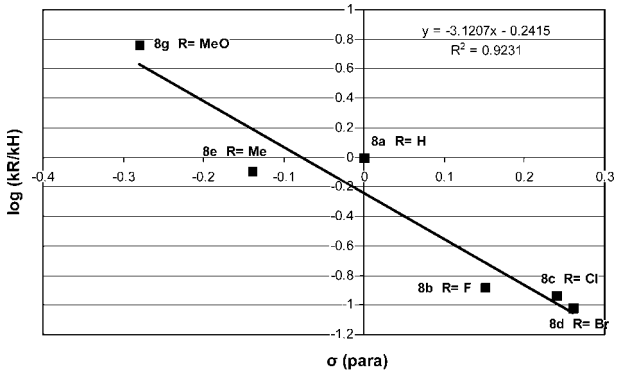


FIGURE 2. Hammett plot for spirodioxyselenuranes **8a**−**e**,**g**.

treatment of the spirodioxyselenuranes also produced a negative slope, but of considerably greater magnitude ($\rho = -3.1$).

We hypothesize that the mechanisms for the catalytic cycles of the aromatic analogues **7** and **8** are similar to those reported previously for the aliphatic compounds **3** and **4**, respectively (Schemes 2 and 3). Since the steps involving substitution or reductive elimination by the nucleophilic thiol would be facilitated by a more electrophilic selenium center, they would be enhanced by electron-withdrawing substituents. On the other hand, steps where Se(II) is oxidized to Se(IV) would be expected to proceed more rapidly in the presence of electron-donating substituents that are capable of stabilizing the development of increased positive charge on the selenium atom. The negative values of ρ indicate that the latter is the case, supporting the hypothesis that the oxidation of selenium comprises the rate-determining step in both series of compounds **7** and **8**. The large negative value of ρ in Figure 2 reveals that the oxidation of the selenide to its corresponding selenoxide in Scheme 3 is particularly sensitive to the nature of para substituents. Moreover, the kinetic plots of the assays for catalysts **8** (see the Supporting Information) show positive *y*-intercepts, indicating anomalously high rates of formation in the early stages of the reaction. This is consistent with the rapid consumption of the catalyst (10 mol %) and concomitant conversion of thiol to disulfide in the first two steps in Scheme 3, prior to the rate-determining oxidation of Se(II) to Se(IV), and eventual regeneration of the catalyst. The linear (zero-order) disappearance of thiol and formation of disulfide with time (see the Supporting Information) also supports the oxidation of Se(II) to Se(IV) as the rate-limiting step in both series of compounds.

Finally, it is especially noteworthy that the methoxy-substituted spirodioxyselenurane **8g** displays catalytic activity superior to that of all of the other aromatic compounds in Table 1 and approaches that of the aliphatic prototype **4** (see entries 10 and 17 in Table 1). Thus, the exploitation of substituent effects has enabled us to emulate the high activity of the aliphatic species **4** while circumventing the generally greater toxicity associated with aliphatic selenium compounds.

Experimental Section

Compounds **7b**−**g** and **8b**−**e**,**g** were prepared by variations of the procedures used earlier for the preparation of **7a** and **8a**, respectively.[13a] Sample procedures are described in the Supporting Information, along with characterization data for new compounds.[22]

Catalytic activity was measured by adding the catalyst (10 mol %) to a mixture of 28% hydrogen peroxide (0.035 M) and redistilled benzyl thiol (0.031 M) in dichloromethane−methanol (95:5) while maintaining the temperature at 18 °C. The reactions were monitored by HPLC analysis, using a UV detector at 254 nm and a reversed-phase column (Novapak C_{18}; 3.9 × 150 mm), with naphthalene (0.0080 M) as an internal standard. Acetonitrile−water was employed as the solvent (gradient: 60:40 to 80:20 over 15 min with a flow rate of 0.9 mL/min). Each $t_{1/2}$ value in Table 1 is the average of at least two runs. Kinetic plots are provided in the Supporting Information.

Acknowledgment. We thank the Natural Sciences and Engineering Research Council of Canada for financial support.

Supporting Information Available: Typical procedures, characterization of new compounds, including ^{1}H and ^{13}C NMR spectra, and kinetic plots for the results summarized in Table 1. This material is available free of charge via the Internet at http://pubs.acs.org.

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(22) Among compounds **7**, **8**, **11**−**13**, and **16**, only the unsubstituted derivatives (series **a** in Table 1) and diselenide **11d** have been reported previously. For the unsubstituted compounds, see ref 13a; for **11d**, see: Vafai, M; Renson, M. *Bull. Soc. Chim. Belg.* **1966**, *75*, 145.