Natural Product Phenolic Diglycosides Created from Wildfires, Defining Their Impact on California and Oregon Grapes and Wines

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ABSTRACT: Forest fires produce malodorous phenols, bioaccumulated in grapes as odorless phenol glycosides (mono- to tri-), and produce unpleasant "smoke tainted" wines when these complexes are transformed by glycosidases in saliva. Metabolomic analyses were used to further understand "smoke taint" by quantitating marker phenolic diglycosides via UHPLC separations and MS/MS multiple reaction monitoring. A collection of grapes and wines provided data to forecast wine quality of grapes subjected to wildfire smoke infestations; the analytics used a panel of reference compounds (1–6). Overall, eight different Vitis vinifera varietals were examined from 2017–2021 vintages involving >218 distinct samples (wines and/or grapes) from 21 different American Viticulture Areas. Results acquired allowed correlation of phenolic diglycoside levels as a function of grape cultivar, varietal clones, and intensity of wildfire smoke. Baseline data were tabulated for nonsmoked samples (especially, Cabernet Sauvignon having a sum 1–6 of <6 μg/L) and then compared to those exposed to six other levels of smoke. Outcomes established that (1) analyzing paired samples (bottled wines versus smoke-exposed grapes) can provide diagnostic metabolomic data, (2) phenolic diglycosides are stable in wines aged for >2.5 years, and (3) major gaps exist in our current understanding of this pool of metabolites.

Prior to 2016 and 2017, wildfires were not common near vineyards in California and/or Oregon. Since then, the increasing occurrence of fires during grape harvest now presents a persistent problem. Current understanding on the impact of such fires on wine quality has come from decades of research in Australia, especially at the Australian Wine Research Institute (AWRI).1 Overviews describing mechanisms on how smoke taint can arise and impact on fine wines can be found in a recent American Chemical Society periodical2 and from a timely comprehensive review.3 Grapes and their vines physically survive wildfires, but the full impact from smoke and ash remains hard to assess.4 The viticulture and enology communities are concerned that wine quality faults, sometimes termed "smoke taint", resulting in undesirable tastes such as "ashy", "bitter", or "smoky",5–6 are difficult to quantify by common analytical methods.5–7 Also analytical chemical data can be hard to correlate with sensory evaluation results.9

It is currently difficult to accurately assess the extent of quality reduction on grapes or wines exposed to smoke and/or ash from small brush or large forest fires.10 Damage to smoke-exposed grapes originates from volatile phenols putatively produced by pyrolysis of plant polyphenolic compounds (lignins) during wildfires. More than 500 volatile odoriferous compounds are contained in wood-derived smoke,11 and approximately 34 volatile phenols (Figure S8, Supporting Information) have been identified from wildfire smoke or barrel toasting or released by grapevine leaves. It is important to recognize that elevated levels of scented phenols can be detected for premium wines aged in toasted barrels; these can be up to 100 ppb for guaiacol and 20 ppb for 4-methylguaiacol.3 In this study, we sought to reinvestigate the use of bioanalytical chemistry tools to quantitate levels of a subset of sensory active phenols in grapes and wines. The goal was to gather a relatively large data set to rapidly forecast quality in fine wines made from grapes exposed to wildfire smoke. In 2003, the AWRI7 provided some of the first indications that grapes covered with wildfire smoke could result in wines significantly reduced in quality. In the subsequent decades many academic laboratories13–15 and currently over 10 corporate groups have intimated that insights on quality can be obtained by measuring levels in wines of

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Concurrent with these efforts was the approach to estimate
total volatile phenols by subjecting samples to hydrolysis (acid
or enzymatic) followed by GC-MS analysis. Next-
generation methods featured optimized hydrolyses via 1.0 N
HCl and 1.25 N H2SO4, or alternatively HCl at pH 1.5 over
4 h at 100 °C. The information provided by these two
approaches, while useful, are of diminished value; the
concentration levels of free volatile phenols are often very
low, and data obtained for total volatile phenols involve
indirect determinations.

The project design (Figure 1) emphasized direct analyses, at
the ppb level, of bound glycosylated volatile phenol
compounds [denoted herein as phenolic diglycosides (PDs)].
Determination of their concentrations in grape juice or wine
was done in this study using multiple reaction monitoring
(MRM) data acquired from a triple-quadrupole mass
spectrometer. The goal was to accurately measure the levels
of volatile phenol complexes, themselves being odorless and
sometimes tasteless, that accumulate in grapes from forest
fires. The use of coupled HPLC-MS/MS to qualitatively
estimate levels of bound PDs produced from volatile phenols
in smoke was introduced in 2010 by the AWRI. Significantly,
in 2013 the AWRI team stated, “The quantitation of phenolic
glycosides in grape homogenates and wines provided a
significant improvement in the ability to distinguish between
non-smoked (clean) and smoke-exposed samples compared to
the existing guaiacol (and 4-methylguaiacol) analysis.” The
logic of analyzing for bound PDs was also consistent with a
2017 finding that a variety of volatile phenols landing on grape
skins rapidly diffuse through the waxy cuticle and, once
inside the berry, they are spread throughout the four grape
berry storage sites. Furthermore, the phenols are rapidly
transformed into bound PDs by promiscuous uridine-
diphosphate glycosyltransferases (UGDTs). It has also
been postulated in a recent review that only volatile phenols
function as substrates for the catalytic proteins of the UGT72
family in grapes. In view of these developments, bound PDs
can be hypothesized as stored in the estimated percentages in
Figure 1 extrapolated from the sugar levels tabulated in a 2011
review.

A conclusion, based on the above discussion, is that the best
way to measure smoke-derived phenols in grape juice and/or
wine is by their direct quantitation, accompanied by an
efficient sample workup. Thus, commercially available stand-
ards, plus deuterated analogues, were utilized herein and
greatly improved the quantitative recoveries obtained. This
approach also facilitated optimizing HPLC retention times
(t_R)
and provided confirmation of molecular structures being
analyzed during MS/MS runs. Another rewarding experimental
design element involved side-by-side analyses of bottled wines
(ideally nonsmoked and even barrel aged) versus new
production wines and/or fresh grapes and whenever possible
including paired legacy and new samples obtained from the
same vineyard. Shown below is that the latter step provided a
definitive way to estimate concentrations of baseline analytes
compared to those potentially elevated from an environmental
smoke episode.

While the primary focus in this work was on Cabernet
Sauvignon, other varietals examined included Merlot, Cabernet
Franc, Malbec, Pinot noir, Syrah, Grenache, and Zinfandel.
Using the breadth of data accumulated, new insights can be
contributed to directly re-examine current practices and further
probe current uncertainties in issues as follows. (a) A current
view offered by the AWRI and UC Davis (UCD) is that the
best way to get an initial insight on potential smoke damage to
wine quality is to do a microfermentation of grapes even
though there are published results that indicate this step may

Figure 1. Workflow to harness the biological–chemical mechanisms producing the six phenolic diglycoside (PD) marker compounds 1–6. Wildfire-created phenols are absorbed into grapes via uridine-diphosphate glycosy transferases (UGDTs), then stored in grapes as PDs, and can be released into the must during winemaking. This project builds on previous analytical strategies to estimate smoke taint wine faults; unlike many past studies, the work engaged in a direct measurement of PDs in grapes and wines.
not be needed.18 (b) Many are hopeful that levels of baseline bound PDs in grapes from Australia versus those of the U.S. West Coast are comparable, but such data have not been completely published.1 (c) Levels of baseline bound PDs can vary as a function of American Viticultural Areas (AVAs) and cultivars,24 but there are knowledge gaps for U.S. West Coast varietals. (d) The accumulation and fate of bound PDs in wildfire-exposed grapes can vary during winemaking and aging,25,26 yet this idea has only received limited testing. (e) Knowing the exact levels of bound PDs will inform production staff about useful next steps, including sensory evaluations and remediations,4 and accumulation of an expanded understanding of bound PDs needs to be considered. (f) The original panel of 12 marker bound PD compounds14 subsequently was pruned to six by deleting the pentose-containing analogues (see Schemes 1 and S1, Supporting Information), yet a further re-evaluation might be desirable given the putative recent discovery of 31 phenolic diglycosides in Napa Cabernet Sauvignon grapes to wines26 and the continuing lack of studies to thoroughly characterize impactful PDs present in smoke-infested vineyards.27

Overall, the goal of this project, designated herein as the Santa Cruz Campaign (SCC), was to gather grape and wine samples from California and Oregon vineyards proximal or distant to natural fires spanning 2017–2021. As noted above, unfermented grape juice and their finished wine were targeted for quantitative analysis of six bound PD standards (1–6) by HPLC-MS/MS. This approach would build on previous outcomes, pioneered in Australia, to interrogate the accumulation and fate of bound PDs in grapes exposed to forest wildfires. Importantly, the findings, to be shared with the community from such a study, should further extend the utility of (a) extensive data gathered in Australia (over >15 years by the AWRI) on smoke- and non-smoke-exposed white and red wines,1,14 (b) the emerging results being accumulated from the Okanagan Valley, Canada,15 and (c) the fascinating data recently reported for 2018 Cabernet Sauvignon samples by a University of California, Cooperative Extension (UCCE) team on wildfires in one Napa County (NC) and 13 Lake County (LC) vineyards.10 Examined herein, from 2017–2021 vintages, were over 200 red grapes and/or wines encompassing multiple varietals from several AVAs on the U.S. West Coast.

■ RESULTS AND DISCUSSION

Merging Viticulture Circumstances with Bioanalytical Method Development. The major wine country regions of the U.S. West Coast are divided into distinctive AVAs, with 139 in California and 22 in Oregon. Distinctive wine qualities, unique to these wine-growing regions, are under threat from the steady stream of wildfires, yet these may not equally impact all vineyards in an AVA. Significantly, exposure of vineyards in Australia to artificial smoke for just a few hours after veraison caused smoke taint. A discussion provided above sought to underscore the power of using bound PD concentrations to gain insights, independent of directly measuring smoke densities in wine-growing areas; however very few such general U.S.-based results are available up until now. This study sought to fill that gap by examining how bound PDs might vary as a function of cultivars and AVAs. The obvious next step was to engage in a broad-based pan-examination of grapes from...
selected vineyards in California and Oregon. Outlined below are seven categories of smoke impact outcomes based on the 1−6 concentrations measured in U.S. West Coast grapes and/or wines.

**Overview of Samples and Strategies Employed.** Fortunately, the SCC team was able to acquire duplicate samples of 13 of the 14 Cabernet Sauvignon 2018 wines described above, and concentrations were determined for the six classic bound PD chemical markers 1−6. This was an essential action to validate measurements done at SCC and enabled direct comparison with the same data measured at AWRI (Figure S1, Supporting Information). Eventually, seven different grape varietals were examined from 21 California or Oregon AVAs divided into 218 samples consisting of 167 postfermented wines, 23 microfermentations, and 28 grape juice samples.

A cornerstone discovery previously published described using 12 bound PDs to evaluate a smoke-impacted Australia 2009 Cabernet Sauvignon wine. The conjugates used consisted of phenol and five other substituted phenols fused by a glycosidic bond to gentiobiose, rutinose, or pentosylglucose (Schemes 1 and S1, Supporting Information). The study described below favored employing gentiobiose- and rutinose-containing bound PDs based on (a) the commercial availability of 1−6 and their deuterated analogues and (b) that these compounds could be resolved by HPLC. The process to quantitate 1−6 in complex wine samples was also facilitated by the three strongest negative-ion collision-induced dissociation (CID) mass transitions observable for each compound using collision energies of 20 to 42 V accompanied by the side-by-side comparison of the fragment ion m/z peaks for the proton- versus deuterated-containing analogues. Thus, it was possible to confidently decipher (Figure S7, Supporting Information) the MS/MS-created network of m/z values observed.

Early on, there was a concern about a potential complication implied by discoveries reported during measuring the qualitative levels of phenolic glycosides of a smoke-impacted 2017 Oakville (Napa County) Cabernet Sauvignon. That work, guided by negative-ion high mass accuracy MS/MS analyses, included a somewhat unusual passage, “it is notable that syringol- and guaiacol-diglycosides do not predominate as previously reported”, in the Australian work. Curiously, none of the 15 PDs provisionally identified therein included compounds 1−6. Also, in contrast to the 2019 study, were findings from a 2020 study of 14 Cabernet Sauvignon grapes and wines from Napa and Lake Counties variably impacted by fires. In this report, all compounds 1−6 were detected in varying levels derived from MS/MS data. Reported below are several sets of important proof-of-concept outcomes obtained here using MS/MS data and compound standards.

**Quantitation of Bound Phenol Diglycosides in 2018 Cabernet Sauvignon from 14 California Vineyards (Lake and Napa Counties).** These wine samples were re-examined from vineyards exposed to varying levels of wildfire smoke (three major fires: Ranch, River, or Snell). The UCCE group harvested the grapes and carried out microfermentations followed by bottling. Interestingly, all of the vineyard locations were proximal to high-impact Ranch and Snell fires as well as to eight others that were of low or no impact. Analytical results, obtained at three different laboratories on identical samples, are shown in Figure 2. The data measured at AWRI are indicated as blue bars (harvest I = August 17) or as orange bars (harvest II = September 17), and data from SCC as gray bars (harvest II). To get an additional perspective, SCC sent...
three samples (harvest II) to ETS Laboratories, and the data received are indicated as yellow bars (harvest II). Additional overall insights came using data for total bound PDs (ppb) measured for the 2018 collection (Figure 2) alongside the related 2019−2021 assemblages. The 1−6 ppb totals observed over the 2018 to 2021 vintages spanned 0 to 400 ppb. Thus, now proposed is that smoke infestations for California Cabernet grapes or wines, independent of cultivar or AVA, can be distributed into the following categories: (a) unsmoked (or baseline) < 6 ppb, (b) light = 6−30 ppb, (c) modest = 31−100 ppb, (d) significant = 101−200 ppb, (e) elevated = 201−300 ppb, (f) substantial = 301−400 ppb, or (g) severe > 400 ppb. Applying these categories allows the 2018 wines (Figure S1, Supporting Information) to be characterized as (i) one vineyard (#14) as unsmoked, (ii) one vineyard (#12) with light smoke, (iii) eight vineyards (#3–#9, #13) with modest smoke, (iv) the remaining four (#1, #2, #10, #11) with elevated or substantial smoke impact, and (v) no vineyards with severe smoke levels.

Translating quantitative bound PD data to estimate an absolute sensory impact on wines is not always straightforward even when the evaluators are trained tasters. A confounding issue is that the marker compounds measured, as well as any related PD analogues, are odorless. They can only be noticed during a tasting after in-mouth breakdown of PDs occurs by enzymes or bacteria present in human saliva, which cleave the glycosidic bond and releases the offensive-smelling phenols. Consequently, sensory evaluation assessments of wine quality for potential smoke impact can be imprecise because biochemical-based organoleptic variations occur among individuals. Clearly, it should be easiest for a panel to rank wines that are smoke-free or heavily smoke impacted. A deeper understanding was sought on the relationship of smoke impact from the large Ranch and River fires (July 17) and the sizable Snell fire (September 8) correlated to the two harvest dates (above as I and II). It is reassuring that the four vineyards (#1, #2, #10, #11) designated as elevated or substantial in concentrations of bound PDs appeared to be within range of the Ranch or River fire smoke zones. Alternatively, a tally of the ppb total for vineyards closest to the Snell fire, #12 = 15−18 ppb and #13 = 31–38 ppb, indicated the smoke impact was light to modest. Significantly, the ppb values did not change from harvest dates I to II. Thus, it is tempting to conclude that modest smoke infestation to these vineyards came from the more distant Ranch or River fires. It is clear that the total PD (ppb) data can provide some powerful insights.

Comparing Grape Juice and Wines for Bound Phenol Diglycosides in 2021 Cabernet Sauvignon from 14 California Vineyards (Lake and Napa Counties). To date, no publications have compared directly quantitative bound PD levels in Cabernet Sauvignon harvested from multiple vineyards after veraison followed by a few days of grape maceration and then fermentation. This task was the focus of another key step in the project design. Each week, from September 1 to 22, grapes were gathered by the UCCE group from the same 14 vineyards that they picked in 2018. The grapes (500–1000 g) were sent to the SCC and kept refrigerated prior to destemming; then maceration was carried out for approximately 5 days at <60 °C, to inhibit premature

Figure 3. (a) Quantitation of 1−6 in 2021 Cabernet Sauvignon unfermented grape juice (blue bars) and wine (red bars) from LC and NC vineyards exposed to varying levels of wildfire smoke. (b) Locations of vineyards versus 2021 named fires.

UCCE 14-member panel assembled to evaluate all 14 of the 2018 Cabernet Sauvignon wines. Recall, presented above, that these 14 wines were divided into five categories (i−v) based on bound PD data. In contrast, the sensory panel divided the wines into three general types: five wines had “no” to “barely perceptible defects” and only one had “serious defects”.

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fermentation of juice. A six-day minifermentation of the grapes on the skins (inoculated with W-15 yeast) proceeded uneventfully to completion, and the liquid wine samples were collected. The bound PD concentrations were measured (Figure 3) for both the grapes (purple bars) and the wines (red bars). The total concentrations (Figure S2, Supporting Information) of marker compounds 1–6 ranged from 6 to 36 ppb and were similar to that measured for their respective wines, ranging from 5 to 36 ppb. Significantly, the small differences in the individual and total bound PD ppb data for matched grapes versus wines demonstrate that examining juice from small grape batches using a five-day maceration to extract the marker compounds prior to a large harvest accurately forecasts any potential negative impacts to be expected in the final wine from wildfire smoke infection. This observation is also consistent with outcomes, though overlooked by many, reported in 2008.18 Significantly, this classic 2008 publication demonstrated, through measurement of total guaiacol and 4-methylguaiacol levels on artificially heavy smoked Australia Merlot grapes, that levels of these two compounds peaked in ppb concentrations after a seven-day maceration and their concentrations did not increase after a subsequent fermentation step. Overall, based on the seven categories discussed above, only one of the grapes or wines (#9) from the 2021 vineyards exhibited high enough levels of bound PDs that would be considered somewhat worrisome. Using the categories discussed in the preceding section justified dividing the 2021 wines and their respective grapes into (i) one vineyard (#14) as unsmoked, (ii) 12 vineyards (#1–#8, #10–#13) with light intensities, and (iii) one vineyard (#9) with modest levels. These assessments are consistent with the low or no wildfire smoke impact from the four 2021 named, very small wildfires. This circumstance also needs to be compared to the data from 2019 (Figure S3, Supporting Information) discussed below, where all 14 vineyards exhibited bound PDs < 5 ppb. These data are further in great contrast to the situation noted above for 2018, where four vineyards (#1, #2, #10, #11) were designated as having elevated or substantial smoke impact, while only vineyard #14 was in the unsmoked category. Closer inspection of the bound PD ppb levels of grapes versus wines for the two 2021 vineyards with the greatest totals is informative including #9 = 36.2 ppb (grapes), 36.3 ppb (wines) and #11 = 33.5 ppb (grapes), 27.4 ppb (wines). For both these 2021 samples, the highest individual total PD concentrations were modest and mostly came from two compounds as follows: vineyard #9: 2 = 15.5 ppb (grapes), 15.4 ppb (wines), 5 = 10.1 ppb (grapes), 11.3 ppb (wines); versus vineyard #11: 2 = 15.8 ppb (grapes), 12.5 ppb (wines), 5 = 11.9 ppb (grapes), 10.3 ppb (wines). These changes from grapes to wines are minor and indicate bound PDs initially accumulated in grapes in modest amounts are stable during and after fermentation. Additional data discussed below will show that bound PDs accumulated in grapes at elevated amounts are also stable during and after fermentation.

Assessing the Long-Term Stability of Bound Phenol Diglycosides in Red Wine. Examining the long-term biosynthetic stability of bound PDs present in modest to significant amounts in finished wines represents another important undertaking. While such insights could not be obtained from the 2021 Cabernet Sauvignon data, relevant conclusions came from comparing changes for the 2018 Cabernet Sauvignon samples. The key was to compare data sets measured at AWRI in November 2018 on the wines just bottled (without barrel exposure) versus those measured in August 2021 by SCC after >2.5 years of bottle aging. The most relevant data (Figure S1, Supporting Information) are from the four most smoke impacted vineyards showing that with time the total levels uniformly slightly decreased for all: #1 = 250 ppb to 223 ppb, #2 = 310 ppb to 291 ppb, #10 = 275 ppb to 241 ppb, and #11 = 400 ppb to 351 ppb. However, these results must be considered as provisional pending their retesting at the AWRI to assess for systematic error differences. Nonetheless, the relative similarity of these data indicates that compounds 1–6 are robustly stable in wine over several years. This is in agreement with a past report showing that bound PDs present at significant levels at bottling were approximately the same in an Australia Cabernet Sauvignon after <5 years of bottle aging.
Multiyear Quantitation of Wines from 14 California Vineyards (Lake and Napa Counties) to Define Baseline and Elevated Levels for Bound Phenol Diglycosides.

From 2017 to 2021, the wildfire smoke impact on the 14 vineyards was quite variable. Four of the 2018 vineyards (#1, #2, #10, and #12) were described above as being significantly impacted by wildfire smoke because of their high levels of bound PDs 1–6 (223 to 351 ppb, Figure S1, Supporting Information). By contrast, low levels were measured by the AWRI for 2018 vineyard #14 (2 ppb), and this provided baseline concentrations expected for a clean California Cabernet Sauvignon wine. By contrast, the AWRI background survey database of non-smoke-exposed Australia Cabernet Sauvignon indicated that clean grapes have the sum of 1–6 of up to 24.6 ppb. Initially, these differences seemed troubling; so, more insights were sought by collecting data from 14 vineyards of the 2019 vintage, where none of the named fires had smoke impact. The total level of bound PDs < 5 ppb was

![Figure 5](image-url)  
Figure 5. Fate of 1–6 from maceration to fermentation by their quantitation in 2021 Cabernet Sauvignon and Zinfandel grapes (purple bars) and wines (red bars). Samples were from vineyards in LC (#9, #11, #12) and ED (ED-1, ED-2, ED-3).

![Figure 6](image-url)  
Figure 6. Comparison of 1–6 in Pinot noir wine samples from SCM grapes harvested in 2019 or 2020. Matched samples were obtained in 2019 and 2020 from vineyards #9 to #12. The data from all 2019 vintages provide baseline data for unsmoked samples, total 1–6 = 1–5 ppb.
observed from each of the 2019 vineyards. These data, plus those of the 2018 vineyard justifi
ed the present assignment of the baseline of $1^{-6}$ as <6 ppb for an unsmoked California Cabernet Sauvignon.

Considering the comparative data collected from 2019 to 2021 harvests from the 14 vineyards provided a way to expand on insights considered above. Also, another important wine sample, from vineyard #14 harvested in 2017, was provided by UCD, and as discussed above, the 2019 publication putatively reported 31 bound PDs. Accordingly, comparative total $1^{-6}$ concentrations obtained during this work for selected wine samples of four key vineyards (#2, #11, #12, and #14) were scrutinized (Figure 4) for the 2017–2021 Cabernet Sauvignon vintages. First, as noted above, all 14 vineyards for 2019 possessed bound PDs < 5 ppb, as did #14 in 2021. Second, among the collection, vineyard #14 was observed to have light smoke infestation in only two out of the five years: 2017 = 30 ppb and 2020 = 13 ppb. Third, light smoke infestation was also concluded for 2018 vineyard #12 (15 ppb): light to modest for 2020 vineyards #2 (21 ppb) and #11 (49 ppb) and light for 2021 at #2 (13 ppb), #11 (33 ppb), and #12 (8 ppb). Finally, large totals of bound PDs were observed at only three of these selected vineyards in varying years: for 2018 at #2 (291 ppb) and #11 (351 ppb) and for 2020 at #12 (408 ppb).

The situation for the 2017 vineyard #14 needs further comment. Five of the six marker compounds were observed, but at relatively low ppbs (Figure 8), and 6 was not detected. This pattern was mirrored in the miniscule levels of 6 measured from vineyard #14: ppb = 0 in 2018, 2019, and 2021 and 0.7 ppb in 2020. Why marker compounds 1–5 were

Figure 7. Quantitation of $1^{-6}$ in six different wine varietals (Merlot, Cabernet Franc, Malbec, Syrah, Oranache, and Zinfandel) from 2018, 2019, 2020, or 2021. The samples were obtained from vineyards in SCM, NC, SON, and AM.

Figure 8. Future prospects prompted by analysis of a 2017 Cabernet Sauvignon using wine made from mildly smoke-impacted grapes of vineyard #14 (see Figures 2 and 4). This analysis used EIC to visualize the presence and retention times of several compounds having a molecular weight of 432.2 Da. The run shown involved MS CID at 477.2 = cluster [MeFA (formic acid) − H]$^-$ producing the negative ion fragment at $m/z$ = 307.1. The LC-MS/MS trace shows standard 3 (red) and coeluting major analyte peaks (green) including 4.3 min = 3 and two other C$_{9}$H$_{18}$O$_{11}$ isomers at 4.2 and 5.0 min.
observed by SCC and not by the UCD team is discussed below.\textsuperscript{26} It may be contended that ppb levels of 6 are not of diagnostic value because of their scant concentrations in grapes exposed to light or modest wildfire smoke. The ppb concentrations of 6 and also that of 4 only become informative for high levels of smoke exposure such as elevated to severe (Figure S10, Supporting Information). It is now suggested that using 6 and 4 should be abandoned for U.S. cultivars, while still useful for Australia vineyards (Figure S6, Supporting Information). Finally, the present data sets for the five-year period, 2017−2021, show that only a few of the selected 14 vineyard sites, as a function of year (i.e., 2018 #2 and #11 and 2020 #12), would have been suitable candidates to use bound PDs as tools to track chemical biology mechanisms occurring during winemaking involving grapes exposed to significant wildfire smoke.

**Quantitation of Bound Phenolic Diglycosides in Santa Cruz Mountain (SCM) Cabernet Sauvignon.** Participants in the SCC supplied matched sets of wines from 2020 (CZU fires) and 2019 (no impactful fires) (Figure SS, Supporting Information). The present analyses involved vineyards outside the CZU fire epicenter, and it appeared the level of new smoke at these sites was not intense. In 2019, the bound PD totals of 1−6 ranged from 3 to 5 ppb at all seven vineyards, which spanned about 30 miles. These results gave another set of baseline data of 1−6 at <6 ppm expected for normal Cabernet Sauvignon grapes. Even though all of the 2019 wines analyzed were in toasted barrels, this did not affect the assessment of an accurate baseline total and underscores the circumstance that PDs are not biosynthesized in barrels during toasting.

Elevated levels of bound PDs > 30−100 ppb observed for some 2020 wines suggests that they should be placed on a watch list for continuing sensory evaluation. In this regard, three of the seven vineyards from 2020 were in this range, including SCM-17 (33 ppb), SCM-18 (48 ppb), and SCM-20 (36 ppb). Furthermore, the wines from the other four vineyards cannot be designated as absolutely clean and included SCM-16 (12 ppb), SCM-19 (10 ppb), SCM-21 (13 ppb), and SCM-22 (14 ppb). Like the cases discussed above, the ppb levels observed for 4 and 6 were inconsequential. Unfortunately, no samples were available to the SCC from any vineyard located in the high-impact burn zone of the devastating 2020 CZU fire (8/16 to 9/22), which began weeks after veraison and disrupted harvest. Visual inspection of grapes to assess smoke damage may not be useful. For example, Cabernet Sauvignon grapes (Plate S1, Supporting Information) hanging below either the scorched or green leaves in the CZU fire zone were obviously exposed to smoke and fire. The grapes were still intact and the plastic netting covering the grapes was not damaged.

**Accumulation and Fate of PDs in 2021 Cabernet Sauvignon and Zinfandel Grapes during Winemaking.** The current understanding of the first steps in translating smoke in vineyards to ruined finished wines is still evolving. Some understandings are based on unassuming logic, including proximity of a vineyard to wildfire(s), fuel source, smoke exposure duration (>1 h or more), age of the smoke, wind patterns, land topography, and heat inversions.\textsuperscript{10,20} Insights based on experimental knowledge show that volatile phenols are mainly translocated through the waxy cuticle of the grape berry\textsuperscript{7} and then converted into bound PDs. Although bound PDs can also be formed through smoke exposure of the leaves, which have a larger surface area versus grapes,\textsuperscript{20,30} their accumulation in leaves is relatively small and the absolute yields of their slow translocation into grape berries are unknown.\textsuperscript{30}

There has been little previous work quantitatively examining the fate of the total PDs from maceration to fermentation in North American grapes.\textsuperscript{26,27} Data were gathered to address this circumstance as bound PD concentrations were compared at harvest and after mini-fermentations for grapes from lightly smoke impacted 2021 Cabernet Sauvignon vineyards of NC and LC. Further work included a mini-time-course evaluation of 2021 Cabernet Sauvignon grapes during maceration. Interesting results (Figure 5) were obtained even though the smoke levels at vineyards varied from light to modest. Among this assemblage, there were two vineyards having bound PDs > 31 ppb: #9 (total ppb grapes/wines = 36.2/36.3) and #11 (total ppb grapes/wines = 33.5/27.4). Another three vineyards had bound PDs ≈ 20 ppm: #5 (total ppb grapes/wines = 19.7/18.1), #7 (total ppb grapes/wines = 22.0/19.3), and #13 (total ppb grapes/wines = 18.1/18.3). Overall, the total ppb concentrations of bound PDs in modestly smoke-impacted grapes did not change much during fermentation. Likewise, the approximate buildup of bound PDs appears to be rapid early on with additional small changes occurring at different points during maceration or fermentation for vineyards #9 and #11. However, this very small data set needs more entries to fully reveal what happens during fermentation.

Next, it was important to expand the present data set by including vineyards severely exposed to smoke. Fortunately, the UCCE provided samples of 2021 Cabernet Sauvignon and Zinfandel grapes all harvested from vineyards in El Dorado County (ED), which were intensely smoke-impacted by the Caldor fire. The patterns observed for 500 g grape batches macerated for 5 days compared to the finished micro-fermentations are as follows (Figure 5): Cabernet Sauvignon #ED-1 (total ppb: grapes/wines = 393/436), Zinfandel #ED-2 (total ppb: grapes/wines = 494/413), Zinfandel #ED-3 (total ppb: grapes/wines = 654/821). These data represent some of the most smoke-impacted grapes in the overall present study and also show that their large consortium of bound PDs is also relatively stable and can change modestly up or down during the fermentation. For all these intensely smoke-impacted samples the varying levels of bound PDs 1−3 and 5 seemed to be most diagnostic (data not shown), with three displaying the highest levels (2 = 270 ppb, 3 = 120 ppb, and 5 = 240 ppb), whereas the concentrations of 4 (15 ppb or smaller (with one exception)) are less useful.

It may be postulated that, based on the above results, the pool of bound PDs in modest or large concentrations remains remarkably stable for California grapes during the first steps of winemaking, and this view is in slight variance to some other published findings.\textsuperscript{1,26,27} It is important to reiterate that they are stable across multyears, as discussed above for the 2018 NC and LC Cabernet wines. Finally, an interesting picture is now in hand for using relative bound PD concentrations to assist winemakers in assessing their sensory impacts. If the bound PD concentrations are modest, then it is especially important that the tasting panels include only those individuals possessing robust in-mouth enzymes and/or bacteria capable of rapidly releasing all the volatiles.\textsuperscript{9,21}

**Survey of Pinot Noir Wines of California and Oregon through the Lens of Many AVAs and Their Bound PDs.** Oddly enough, there has only been one comprehensive study
of Pinot noir from California and Oregon evaluating the use of volatile phenols as potential smoke taint marker compounds. Finished wines, without barrel aging, were examined from five vintages (all Dijon clone 667) of 15 vineyards in eight AVAs. The calculated sum of seven phenols analyzed for the 2019 vintage revealed free = 6.3 ppb and total = 16.4 ppb; guaiacol was stated to vary by AVA as free = 1.2–2.3 ppb and total = 8–12 ppb. A strength of this study was that these baseline measurements were similar across the AVAs. However, the usefulness of this work is lessened because (a) no comparisons were made to smoke-impacted wines; (b) there were no data or discussion on bound PDs; and (c) just one clone was included in the study. The present work sought to extend the potential groundbreaking effort represented in that 2021 publication by exploring eight clones of Pinot noir grapes from 18 vineyards and eight AVAs in California and Oregon.

The present comparisons began with tallying the bound PD disaccharides 1–6 in Pinot noir wine samples from SCM grapes harvested in 2019 and 2020 from 10 distinct vineyards. Matched samples were obtained in 2019 (no fires) and 2020 (CUZ fires) from vineyards #SCM-9 to #SCM-12 (Figure 6). The data from all 2019 vintages provided baseline sums from MS/MS quantitation of 1–6 samples of 1–5 ppb, representing unsmoked wines. The clones embodied in the baseline wines included Pommard, Martini, Mt. Eden, and Dijon 37 (plus multiple wines were blends of four Dijon clones). Interestingly, one of the 2020 wines, #SCM-16, could also be categorized as unsmoked (bound PDs < 5 ppb); some of the samples had bound PDs of 39–66 ppb and are classified as modest, and another set of samples had bound PDs of 16–28 ppb and are classified as light. As a final point, the concentration of marker compound 4 was uniformly 0 ppb for all the wines and, hence, was not useful, and two other compounds, namely, 2 (17–26 ppb) and 5 (12–25 ppb), were very diagnostic in the four samples classified as modest.

The next phase of the data collection involved the comparison of bound PD levels in Pinot noir matched samples from six (2019, 2020, or 2021) Oregon vineyards and from two different (2020) California vineyards (Figure S9, Supporting Information). The singletons from 2020 California grapes (no fires) from Sonoma (SON), SON-1 (clone unknown), and Santa Rita (STR), STR-1 (Dijon clone 667), had 1–6 sums of GVBs of <5 ppm, indicating their grapes were clean.

Turning to the Oregon (OR) samples, it was found that the data for one of the grapes [OR-2-2021 (G)] and several wines (n = 10) were very useful. Seven Oregon wines from 2020 and 2019 vintages had bound PDs of <5 ppm for clones, including Pommard, Dijon 115, Gemini, and Gamay. Three other vineyards for 2020 had bound PDs indicating light smoke taint: #OR-4-2020 = 10 ppb, #OR-5-2020 = 9 ppb, and #OR-6-2020 = 22 ppb. By contrast, the pattern of bound PDs for several of the samples from the vineyard coded as OR-1 was hard to rationalize. The bound PD = 2 ppb for grape juice from OR-1-2021 (G) fit the profile for clean grapes, and the same grapes from OR-1-2020 (G), described as exposed to wildfire smoke in the vineyard, had bound PD = 30 ppb, also consistent with light smoke taint. Alternatively, the smoke taint expected for the OR-1-2020 wine sample was not observed: bound PD = 2 ppb. All of these samples were analyzed during the same run, and the grapes were either stored cold (2021) or frozen (2020) and macerated (<1 day) prior to analysis. Finally, the bound PD values for grapes of OR-2-2021 (G) of 9 ppb seemed elevated compared to the wine from a nearby vineyard, OR-1-2021 = 2 ppb.

Overall, based on this small data set, the smoke impact from wildfires in Oregon Pinot noir wine country may be less pervasive as compared to those in California Pinot noir AVAs. Using the data from this survey it may be proposed that the baseline of bound PDs 1–6 of <6 ppb applies to clean California and Oregon Pinot noir regardless of the clones or AVAs. Throughout this study, it has been shown that the spread in bound PD values provides an accurate distinction between clean versus smoke-impacted wines and grapes, and such differences are also evident here for Pinot noir. However, a potential weakness in the present data set is that no intensely smoke-impacted Pinot noir samples were examined. In the future, it may be predicted that more intensely smoke-tainted Pinot noir wines will be identified with bound PDs > 200.

Quantitation of Bound Phenol Diglycosides in Six Different California Varietals from 2018–2021 Vintages. Wine samples obtained across California AVAs for six additional varietals extended the present study of smoke faults. The additional grapes included Merlot, Cabernet Franc, Malbec, Syrah, Grenache, and Zinfandel (Figure 7). Finished wines, from grapes exposed to varying wildfire smoke levels, were obtained from vineyards in SCM, NC, SON, and Amador County (AM), and they were analyzed for concentrations of the bound PDs (ppb) via LC-MS/MS. Surprisingly, to date, no study has been undertaken to quantitatively examine these cultivars in California, especially Zinfandel, given its rich history. Alternatively, there have been recent results from a study of the Okanagan Valley in Canada on the qualitative (not quantitative) levels of bound PDs from Pinot noir,16 Merlot,16 and Cabernet Franc15,16 where the former two were exposed to artificial smoke.

Zinfandel, the third-leading wine grape variety in California, was evaluated from three AVAs: ED, SON, and AM. Two heavily smoke-impacted 2021 ED vineyard Zinfandel grapes and their wines (bound PD: #ED-2 = 413 ppb and #ED-3 = 821 ppb, Figure 5) were discussed above. No normal Zinfandel grapes could be picked from this AVA to set a baseline value. However, such a calibration was obtained using the data from other samples for which the bound PDs are <9 ppb included #SON-1 (8 ppb), #SON-9 (5 ppb), and #AM-1 (6 ppb) (Figure 7). Two other Zinfandels were clearly exposed to light smoke, as indicated by the bound PD sums for #SON-3 (26 ppb) and #AM-1 (72 ppb). The relative bound PD concentration differences between the baseline and smoke-impacted wines are most influenced by the increase in concentrations of the two rutinosides 2 and 5 and much less from two other rutinosides, 3 and 6, in the following ppb order, respectively: #ED-2 = 116, 271, 46, 55; #ED-3 = 272, 279, 120, 110; #SON-3 = 12, 7, 2, 2; and #SON-4 = 33, 24, 5, 4. Investigations of additional Zinfandel wines from smoke-filled environments are needed to expand an understanding of these preceding trends and confirm that the baseline of bound PDs of <9 ppb for Zinfandel is valid across many AVAs. Overall, the most diagnostic individual chemical markers for Zinfandel seem similar to that of Cabernet Sauvignon and Pinot noir, and its baseline seems slightly higher.

Having a knowledge base on the five California Bordeaux varietals was important. The extensive data obtained for Cabernet Sauvignon was discussed previously. A much smaller set of samples was examined for the three other Bordeaux varietals, Merlot, Cabernet Franc, and Malbec (Figure 7),
while no samples were included for Petite Verdot. Currently, it may be assumed, based on the data for Cabernet Sauvignon, that the bound PD baseline for all California Bordeaux cultivars should be similar and <6 ppb. This proposal is buttressed by the following outcomes. The Merlot samples came from two regions, SCM and NC. One bottled wine, #SCM-19 (bound PD = 0 ppm), was a sample representing an unsmoked entry. The bound PDs of 13–17 ppb for the other three 2020 samples reflected light smoke impacts. The three 2020 Cabernet Franc samples with bound PDs of 5–6 ppb added additional baseline data. The single 2020 Malbec sample (#SON-4) showed higher levels of smoke faults and is considered to contain light levels based on its total bound PD of 26 ppb. In this Malbec and in the modest smoke-impacted 2018 Cabernet Sauvignon, the bound PD level for chemical marker 2 appears to be the most elevated. Finally, across all of the four Bordeaux cultivars examined here, designated as light to modest (i.e., total bound PDs < 101), the least useful diagnostic values are for the rutinoside-containing 3 (bound PD = 0–12 ppb) and the gentiobioside-containing 4 (bound PD = 0 ppb), further highlighting that this pair of bound PDs are not worth analyzing for smoke taint detection in California Bordeaux type samples.

Blends, labeled as GSMs (Grenache, Syrah, Mourvèdre), are popular in California and were also evaluated. Australia Shiraz (aka Syrah) has been shown as having elevated baseline levels of bound PDs, relative to that of other grapes such as Cabernet Sauvignon. Establishing a bound PD baseline level for Syrah in the present study was not straightforward, as only four samples were analyzed. Provisionally, it was concluded that the three samples from non-smoked-impacted 2019 vineyards, #SCM-24 (PD = 5 ppb), #SCM-25 (bound PD = 31 ppb), and 2020 vineyard #SON-25 (bound PD = 17 ppb), could provide a Syrah bound PD baseline of 5–31 ppb. This is higher than the baseline for Cabernet Sauvignon and in agreement with the Australia studies cited earlier. The data for the 2020 CZU fire smoke-filled SCM vineyards, #SCM-24 and #SCM-25, are revealing. The 2020 Syrah from #SCM-24 (bound PD = 132 ppb) was clearly smoke-impacted and had elevated levels of 2, 5, 6, but not 4 (2019 = 0 ppb, 2020 = 5 ppb). Also produced in 2020 from #SCM-25 were Grenache and a Syrah—Grenache blend. Their data showed the expected elevated bound PDs for both the Grenache (bound PD = 155 ppb as significant) and Syrah—Grenache (bound PD = 310 ppb as substantial). For both these samples, two marker compounds were best for quantitation of smoke faults: 2 (Syrah—Grenache = 107 ppb and Grenache = 51 ppb) and 5 (Syrah—Grenache = 89 ppb and Grenache = 36 ppb), and once again, 4 was of minimal importance. Also, examined were two GSMs from the 2020 vineyard #SON-25. Their bound PDs included (a) a Rosé at 6.1 ppb and (b) a barreled red at 21 ppb. Informal sensory evolutions indicated both wines were excellent, and this agreed provisionally with the baseline of <31 ppb for a smoke-impacted Syrah.

New conclusions can be drawn across eight California wine varietals based on the data discussed above. First, chemical marker 4 is a minor bound PD that does not account for high concentrations even in substantial levels of smoked wines. Second, no evidence has been obtained showing that AVA differences influence the baselines of any grapes or vines included in the current study. Last, if the conclusions made of elevated bound PD baseline data are correct for Syrah, then this is in alignment with other data based on elevated baseline levels for Australia Shiraz wines.

Re-evaluating the Important Marker Bound PDs for Red Grapes. It has been demonstrated that directly measured concentrations of bound PDs as markers for assessing wine smoke defects are more powerful than employing data from free or total phenolics determinations. Significantly, minuscule bound PD concentrations are observed in wines, except for Syrah, not exposed to smoke. The quantitation 1–6 herein has extended a strategy introduced in 2010 and used an efficient sample workup. Also, as the project progressed, several potentially confounding issues were evident. First, unproven are that patterns of the bound PDs observed in Australia wines directly apply to smoke incidents in California and Oregon. Second, undefined is the merit of using a larger portfolio of bound PDs such as fenolic pentosyl disaccharides (i.e., 7–12, Scheme S1, Supporting Information). Third, there could be value in expanding the marker compound panel to phenolic monosaccharides and trisaccharides recently observed from California and Canada red grapes. Eventually, further interrogating stable PDs was concluded as most important; these complexes seemed to be accumulated from smoke exposure in the highest relative concentrations.

Comparing the bound PD constituents of three Cabernet Sauvignon smoke-impacted wines provided surprising new insights. These side-by-side outcomes (Figure S6, Supporting Information) included (a) a 2019 Australia smoke-impacted wine (AWRI data for the 10% smoked wine, bound PD total = 246 ppb), (b) a 2018 California smoke-impacted wine (vineyard #11, Figure 2, bound PD total = 351 ppb), and (c) a 2021 California smoke-impacted wine (vineyard #ED-1, Figure 5, bound PD total = 436 ppb). Two compounds dominated the bound PDs for the Australia sample and were 1 (156 ppb, 63%) and 4 (101 ppb, 20%), respectively, while 2 (2%) was the least abundant. Astonishingly, different major and minor bound PD components were observed for the California wines: the 2018 wine contained major constituents including 2 (123 ppb, 35%), 1 (101 ppb, 29%), and 5 (34 ppb, 15%), while 4 (7 ppb, 2%) was very minor; the 2021 wine contained the same major constituents including 2 (110 ppb, 25%), 1 (63 ppb, 14%), and 5 (157 ppb, 36%), while 4 (9 ppb, 2%) was again very minor. A comparison of Australia versus California Cabernet Sauvignon illustrates the vulnerability of assuming that identical cultivars from different continents will have the same baseline levels for nonsmoked grapes (data not shown here but discussed above). Also, in the future, a more effective analysis of the California and Oregon wines should use a different panel of bound PDs versus that defined in the Australia work. This leads to a future goal of optimizing the panel of marker PDs for evaluation of California and Oregon red wines.

A new twist in the present project was to understand the extent that diastereomers may be present in the pool of bound PDs from smoke-impacted California and Oregon red wines. Partial motivation for this additional discovery path came from data reported in 2019 on the Oakville Napa Cabernet Sauvignon and in 2018 on artificial smoke-exposed Canadian Pinot noir. Among the 15 phenolic diglycosides described from the Cabernet Sauvignon, three were isomeric, having a molecular weight of 432.1632 Da and a molecular formula of C_{19}H_{28}O_{11}, but, as previously noted herein, none were identified as 3. Additionally, 3 was observed from the Canadian Pinot noir and also found in all 14 California 2018 Cabernet...
Sauvignon wines. Thus, it became a priority to re-evaluate selected Cabernet Sauvignon samples for molecular weight 432.2 Da isomers to further explore this circumstance. The 2017 Cabernet Sauvignon (#14) was reexamined, and the MS/MS lens was expanded using extracted-ion chromatograms (EICs). The run in Figure 8 involved CID at m/z 477.2 cluster [MoFA (formic acid) − H]− producing MS/MS fragment glycone ions at m/z at 307.1/163.1/103.0 (Figure S7, Supporting Information). The EIC LC/MS/MS trace shows standard 3 (red) and the coeluting major analyte peaks (green): 4.3 min = 3 and two others at 4.2 and 5.0 min (ratios by tR = 0.88, 1.0, and 0.68). Based on the fragment ion network observed, it is postulated that the guaiacol subunit is present and the same 2D structures should apply to the glycones; their structural differences arise due to different glycone configurations at one or more of the 10 chiral centers present. Future deeper scrutiny, using additional signature CID fragments, represents an obvious next step to gain stereochemical insights. Also noteworthy is the observation of five other C19H28O11 minor constituents (Figure 8), as putative guaiacol-disaccharide isomers.

A further search for isomers of molecular weight 432.2 Da was expanded through a re-evaluation of the 2017 El Dorado Cabernet Sauvignon wine rated as having severe smoke taint. LC-MS/MS traces were obtained using the CID conditions described above (Figure S11, Supporting Information). The comparative chromatographic profile of the 432.2 Da isomers differed between the two Cabernet Sauvignon wines: 2017 (#14) and 2021 (#ED-1). Interestingly, #ED-1 had peaks at 4.3 min (major) = 3, 4.2 min (minor), and none at 5.0 min. The observation that 3 is predominant (ratio 4.3 min/4.2 min 100/9) in the sample rated as severe and not in the sample rated as light seems significant, but is not easy to rationalize. If the promiscuous UGDTs, responsible for bound PD formation from guaiacol, operate similarly and independently of the grape disaccharide structures, then the mix of the glycone pools within the Cabernet Sauvignon cultivar must differ by smoke composition as a function of AVAs. While the use of 3 as a marker for 432.2 Da compounds is a correct choice, it is still minor (3 = 14%) in #ED-1 versus the presence of the other three important major constituents (see tally above): 1 + 2 + 5 = 76% (and minor for 6 = 9% and inconsequential for 4 = 2%).

It is now suggested, from the data sets produced herein and the additional results from the 2009 Australia Cabernet Sauvignon study,14 that the two least important markers, 4 and 6, for California Cabernet Sauvignon (and other red wines) should be replaced by two pentosyl-containing disaccharides, 7 and 9 (Scheme S1, Supporting Information). Both were observed in relatively high concentration in the Australia Cabernet Sauvignon. Markers 8 and 9 were also tentatively identified by high mass accuracy MS/MS analysis in the 2017 Pinot noir,27 and 9 was potentially the same as hexose-pentose-guaiacol putatively identified in the 2017 Cabernet Sauvignon.27 Our next step will be to obtain synthetic samples of 7 and 9.

### CONCLUSIONS

This study presents some of the first quantitative measurements of PDs bioaccumulated in premium California and Oregon grapes and wines due to wildfire smoke. Strategies of bioanalytics, oenology, and focused collections of grapes from vineyards exposed to varying smoke were merged to create indexes (based on ppb) to estimate the impact of wildfires on wine quality. This will help to guide future wine fault analyses based on merging exact concentrations determined for PDs with less precise qualitative ratings derived from sensory evaluation trials. Metabolomic analyses, guided by UHPLC separations and MS/MS MRM, were buttressed by exploiting a classic panel of six marker bound PDs and their deuterated analogues (1–6). Overall, eight different varietals harvested from 2017 to 2021 were examined, involving >148 distinct samples and their sources encompassing 21 different AVAs. Presented herein are foundational data in the form of ppb sums of the bound PDs for each varietal exposed to approximately seven different levels of natural wildfire smoke.

New understanding has been obtained on ppb variations of 1–6 that can correlate with cultivar, varietal clones (i.e., eight Pinot noir types), and wildfire smoke intensity. Especially useful will be the baseline data tabulated herein for normal grapes, versus those exposed to six other levels of wildfire smoke. It has been demonstrated that reliable PD concentration data can be obtained from a five-day maceration of grapes, so the practice of minifermentations is not needed. It has also been proven that analyses on paired samples (same vineyard block for a barrel-aged wine alongside that for macerated smoke-exposed grapes) provide a rapid strategy to gather metabolomic data for detecting wine quality defects. It is hypothesized that the best analytical results, based on natural product analysis, are derived from grapes exposed to natural wildfires in preference to those subjected to artificial smoke. The outcomes obtained intimate that some baseline data from Australia may not accurately evaluate unsmoked California or Oregon wines. For example, the comparative results for 1–6 totals in clean Cabernet Sauvignon were as follows: Australia (AWRI) 25 ppb, versus California (SCC) < 6 ppb. Discovering the long-term stability of PDs in Cabernet Sauvignon during >2.5 years of bottle aging was significant and greatly contrasts what can occur during a wine tasting. Glycosidases in saliva are known to carry out rapid in-mouth PD biotransformations releasing odorous volatile phenols possessing unpleasant aromas and flavors.

Additional metabolomic evaluations are needed to redesign the current portfolio of PDs used as biomarkers. After surveying many different cultivars, it is recommended that less useful biomarkers 4 and 6 should be replaced with pentosyl-containing complexes, and this needs more study. Further insights could also be derived based on the discovery of additional PD molecules uniquely created by California and/or Oregon wildfires. The quantitation of PD diastereomers, not fully considered to date, should be explored for additional diagnostic patterns. This idea is motivated by the following observations. Using MS/MS selected ion monitoring (SIM) on two different Cabernet Sauvignon grapes/wines in the present panel revealed the presence of seven additional diastereomers of 3, all of which contain the guaiacol residue. Similarly, diastereomers of other candidate biomarkers (Scheme S1, Supporting Information) 8 (n = 3) and 9 (n = 2) were reported in a 2018 study of Pinot noir grapes exposed to artificial smoke.27 However, current MS-only approaches cannot provide a precise molecular structure description, especially for isobaric metabolites. Also, using trial and error total syntheses to get answers has not been successful. Thus, obtaining useful structural information will require a brute force isolation campaign, beginning with wildfire smoke-polluted grapes that are categorized as severe (i.e., PDs >
400 ppb). Future work by SCC aims to accumulate >100 L of such grape juice and launch isolation—structure elucidation work on disaccharide-containing PDs, present in about (or less) 1 \( \mu g/L \) of grape juice. It is very likely that some of these molecules should be isomeric to 1–12 and others should have a structure that has never been described.

### EXPERIMENTAL SECTION

#### General Experimental Procedures

The chemicals used in this work were purchased from VWR, including LCMS grade acetonitrile (CH3CN), methanol (MeOH), isopropanol (IPA), and water (H2O). The LC-MS grade ammonium formate and reagent grade sodium hydroxide (NaOH) were purchased from Sigma-Aldrich. Early development trials were carried out at UC Santa Cruz using a Poroshell 120, Phe-Hex, 2.1 × 150 mm, 2.7 \( \mu m \) column and a Poroshell 120, Phe-Hex, 2.1 mm UHPLC guard column, obtained from Agilent Technologies. Some of the results obtained were equivocal; therefore, the project shifted to runs carried out at SC Laboratories, Santa Cruz, CA, that provided the high-quality data reported in this work. Thus, the entire analytical workflow shifted runs were carried out at SC Laboratories and were the source of all the data reported herein. These analyses used a Raptor ARC-18 100 × 4.6 mm, 2.7 \( \mu m \) particle column and Phenomenex Strata-X 33 \( \mu m \). Polymeric reversed-phase SPE cartridges, bought from Restek and Phenomenex, respectively. Wine fermentations used Lallemand W15 yeast and Lallemand Go-Ferm yeast nutrient purchased from Scott Laboratories, Petaluma, CA.

#### Reference and Calibration Standards

The analysis of bound PDs in grape extracts and wine samples was the cornerstone of the data generation. Reference samples of each of the six bound PD compounds were used for internal standards. These compounds and their isotopically labeled counterparts, all as shown in Scheme 1, were purchased from Toronto Research Chemicals (TCW, Toronto, ON, Canada): syringol gentiobioside (1), syringol gentiobioside-\( d_1 \) (1-\( d_1 \)), phenol rutinoside (2), phenol rutinoside-\( d_1 \) (2-\( d_1 \)), guaiacol rutinoside (3), guaiacol rutinoside-\( d_1 \) (3-\( d_1 \)), 4-methylsyringol gentiobioside (4), 4-methylsyringol gentiobioside-\( d_1 \) (4-\( d_1 \)), 4-cresol rutinoside (5), 4-cresol rutinoside-\( d_1 \) (5-\( d_1 \)), 4-methylguaiacol rutinoside (6), and 4-methylguaiacol rutinoside-\( d_1 \) (6-\( d_1 \)).

Data shown in various Supporting Information figures were based on the 1–6 retention times in all samples. These observations were further supported by negative ion ESI-MS/MS to visualize the samples. Further cleanup via supernatants were treated analogously to that used for the small samples. Further cleanup via filtration was carried out using a Strata-X 33 \( \mu m \) Polymeric reversed-phase SPE cartridge (Phenomenex, Torrance, CA; catalog no. 8B-S100-FB) that was conditioned with 2.0 mL of CH3CN followed by 2.0 mL of H2O. Then, 1.0 mL of the sample was loaded onto the SPE cartridge. The sample was washed with 1.0 mL of 0.1 M aqueous NaOH followed by 2.0 mL of water. The sample was then eluted with 1.0 mL of 40% CH3CN in water and loaded onto the instrument for analysis.

#### Sample Analyses

Samples were processed using an LX-50 UHPLC coupled with a QSight 210 triple-quadrupole mass spectrometer (PerkinElmer, Waltham, MA, USA). A sample (3.0 \( \mu L \)) was injected onto a Raptor ARC-18 with a Raptor ARC-18 guard column. The autosampler temperature was set to 10 °C, and the column oven was set to 30 °C. Mobile phase A consisted of 1.0 mM ammonium formate in H2O with 0.1% formic acid, and mobile phase B consisted of 1.0 mM ammonium formate in MeOH with 0.1% formic acid, with a flow rate of 0.8 \( mL/min \). The pump gradient started with a 0.5 min hold at 30% B, followed by a linear gradient from 30% B to 50% B over the course of 10 min, followed by a 100% B column flush for 5 min, and then returned to 30% B for 2 min to equilibrate.

For MS/MS analysis, phenolic diglycosides were detected in the MRM mode by negative-mode electrospray ionization (ESI). A source temperature of 310 °C was used, with an electrospray voltage of −4200 \( V \), a nebulizer gas of 350 °C, and a drying gas of 120 °C. Parent ions formed from formic acid adduct ions [M + HCOO−]− to the most abundant fragments were used for quantifying and confirming the bound PD. Three mass transitions were used for each compound with the highest mass fragment used for quantification, as it is theoretically the most selective. The CID collision energies varied from 20 to 42 \( eV \) and were optimized by the following standard instrument software’s autotune function.

Listed are the mass transitions and retention times for the six bound PD compounds (see Figure S6, Supporting Information, for the CID fragmentation network): syringol gentiobioside (1) (\( m/z \) 523.3/323.2, 523.2/119.1, 523.3/89.1, 2.49 min), phenol rutinoside (2) (\( m/z \) 477.3/307.2, 477.3/163.1, 477.3/103.1, 2.74 min), guaiacol rutinoside (3) (\( m/z \) 477.3/307.2, 477.3/163.2, 477.3/103.1, 3.21 min), 4-methylsyringol gentiobioside (4) (\( m/z \) 537.4/323.1, 537.4/119.1, 537.4/89.1, 3.75 min), 4-cresol rutinoside (5) (\( m/z \) 461.3/307.2, 461.3/163.2, 461.3/103.1, 4.36 min), 4-methylguaiacol rutinoside (6) (\( m/z \) 491.3/307.2, 491.3/163.2, 491.3/103.1, 4.91 min).

#### Method Validation

All analyte calibration curves were linear with \( R^2 > 0.995 \) with a calibration range of 0.5 to 1000 ng/mL. The method was evaluated by spiking reference standards at two concentration levels (50 and 500 ng/mL) onto three matrix types (wine, fresh juice, and macerated grape juice). Where a blank matrix concentration levels (50 and 500 ng/mL) onto three matrix types was unavailable, matrix blank subtraction was used to assess method performance criteria. Recovery, accuracy, and precision of all analytes were good, with recovery and accuracy within 90–130% and precision (calculated as percent relative standard deviation) less than 5%. While there was a noticeable matrix effect on the absolute response, the presence of the isotopically labeled counterparts for all six bound PDs significantly improved results. For detailed data see Table S1, Supporting Information, establishing limits of detection (LOD) and quantitation (LOQ). Two different standard spike levels of 1–6 involved (medium or high) were used to assess for accuracy percent estimations shown by the 72 entries in Table S1, Supporting Information. Evaluation of precision percent also was based on another set of 72 data entries in Table S1, Supporting Information. Estimating the LOD—LOQ in ppb or \( \mu g/L \) for 1–6 used runs with the deuterium analogues to show differences in Table S1, Supporting Information for direct injection versus SPE cleanup prior to injection. Finally, the uncertainty in percent detection also involved runs with 1–6 deuterium analogues to give the outcomes summarized in Table S1, Supporting Information. Values were established by multiplying the standard deviation of the response of seven replicate matrix spike samples by 3.3 and 10, respectively, and dividing by the slope of the calibration curve.

### Recovery of Bound PD-Analyses

A special analytical run was designed to spike the 2018 wine sample from vineyard #12 (Figures 2
and S1), which was mildly impacted by smoke (total 1–6 = 16 ppb) for a spiking run with known amounts of a mixture containing each standard 1–6. The sample preparation involved adding 50 mL of wine #12 to a conical tube and then adding 25 μL of the standard mix at 100 ppm to this tube followed by vortex mixing. The results from Table 1 show that the percent recovery of standards was excellent. The mixture of Table 1 show that the percent recovery of standards was excellent.

| reproducibility using vineyard #12 (2018 CS) | syringol gentiobioside [ppb] | phenol rutinoside [ppb] | guaiacol rutinoside [ppb] | 4-methylsyringol gentiobioside [ppb] | p-cresol rutinoside [ppb] | 4-methylguaiacol rutinoside [ppb] | total glycosides [ppb] |
|-------------------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| original data (Figures 2 and 15)         | 0               | 9               | 1               | 0               | 56              | 0               | 15              |
| expected increase from spiking          | 57              | 66              | 52              | 57              | 64              | 64              | 359             |
| observed increase from spiking          | 59              | 68              | 56              | 50              | 66              | 59              | 358             |
| recovery %                               | 103             | 104             | 108             | 89              | 103             | 92              | 100             |

### ASSOCIATED CONTENT

#### Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jnatprod.2c00028.

Plate S1, Table S1, Scheme S1, Figures S1 to S11 (PDF)

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Notes

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### DEDICATION

Dedicated to Dr. William H. Gerwick, University of California at San Diego, for his pioneering work on bioactive natural products.

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