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A step on the path in the discovery of new latent fingerprint development reagents: substituted Ruhemann's purples and implications for the law

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Abstract Energies corresponding to optimum geometries of ninhydrin, some of its analogs, the corresponding Ruhemann's Purple analogs and some of the intermediates of the reaction between ninhydrin analogs and amino acids are calculated at Hartree–Fock/6-31G** level of theory. Such a study is significant from a forensic science point of view because of the strong interest in the forensic chemistry and law enforcement communities in developing alternatives to the current generation of ninhydrin-like chemicals for the detection and development of latent fingerprints. In examining our new predictions for the net energetics of the reactions in the formation of substituted Ruhemann's Purples, we find that a fluorine-containing analog is the most thermodynamically feasible reaction. In light of this finding, we suggest further experimental studies to determine the kinetic feasibility of synthesizing the fluorine-containing analog, as well as other similar molecules and to determine their spectroscopic properties.

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Introduction

This work represents an example of the application of quantum chemical methods to Forensic Science. Specifically, it addresses itself to the study of ninhydrin reactions with amino acids as applied to fingerprint identification. One of the methods used for identifying people involved in crimes is the examination of fingerprints found at the scene of the crime [1–5]. There are several different types of fingerprints [5]. Some of them are detected easily on certain surfaces. Others, called latent fingerprints, are harder to detect. Latent fingerprints are invisible prints that are formed by the transfer of perspiration from the finger ridges to an object. Unlike visible prints, to see latent prints, special techniques are needed [6]. One such technique is the use of ninhydrin. When ninhydrin combines with amino acids found in perspiration, it turns purple–blue. The method works as follows: a solution (about 0.6%) is made with ninhydrin powder and a solvent such as ethyl alcohol or acetone. The substance is sprayed onto the surface and prints then appear after a varying amount of time depending on how strong or weak the prints are. The process takes less time if the specimen is heated to 80–100 °C [4, 5, 7, 8].

Ninhydrin (2,2-dihydroxy-1,3-indanedione) was first prepared by Ruhemann, in a reaction that was meant to create a different compound, 1,2-diketohydrindene [9–14]. Soon after ninhydrin was discovered, it was found that many substances turn blue when they are combined with ninhydrin. Finally, in 1954, it was determined to be useful for identifying fingerprints [2]. Ninhydrin is helpful for

finding fingerprints on substances such as paper or wood, where they would not be able to be seen [6].

Forensic scientists are trying to improve the activity of ninhydrin by searching for more effective analogs. While a large literature exists for the synthesis and characterization of alternative compounds to ninhydrin, so far none has been found that offers significant advantages [1, 4]. In this regard, we note the example of 1,8-diaza-9-fluorenone (DFO), which was synthesized in the late 1980s and found to have superior sensitivity compared to ninhydrin [15–17]. However, the compound it produces upon reaction with amino acids in the fingerprint is not as strongly colored as Ruhemann's Purple. Also, Elber, Frank and Almog used semi-empirical quantum chemical computations to suggest several new Ruhemann's Purple analogues that have the potential to fluoresce [18].

In order to help find alternatives to ninhydrin, Petraco et al. [19] have taken the first steps to understand the reactivity of ninhydrin and a model amino acid from a fundamental point of view. Quantum chemistry methods were used to study in detail the reaction energies of the mechanism to form Ruhemann's Purple. This work is a continuation of the latter study and designs a number of analogs of ninhydrin. We compute the energy of reaction of each of these compounds with alanine as a model amino acid, producing substituted Ruhemann's Purples using the Hartree–Fock quantum chemical model.

Computational methods

All computations were performed using the Spartan suite of quantum chemistry computer programs [20]. The structures of each molecule (see below) were optimized at the spin-restricted Hartree–Fock self consistent field (RHF–SCF) level of theory using analytic derivative methods and the 6-31G** basis set. The 6-31G** basis set of Pople is a split valence Gaussian set using one Slater type orbital for the core electrons of each atom, expanded in six Gaussian functions. The valence electrons of the atoms are described by two Slater orbitals, one expanded in a set of three Gaussians, one approximated by one Gaussian. In addition, p-orbitals are set on each hydrogen atom and d-orbitals are placed on each non-hydrogen atom [21, 22]. Cartesian coordinate gradients were optimized to less than 10^{-4} a.u. as convergence criteria. Harmonic vibrational frequencies at the SCF level were evaluated by numerical differences of analytic gradients. All RHF–SCF structures computed in this study are energetic minima.

Results and discussion

Figure 1 shows the net reaction between alanine and the substituted ninhydrin analogues we examined in this paper. Note that only the net reaction to form substituted Ruhemann's Purple is shown. Unsubstituted ninhydrin is labeled structure **1** in Table 1. The side groups for the ninhydrin analogues are indexed by structure numbers **2**–**9** as shown in Table 1. The structures of the Ruhemann's Purple analogues produced in the net reaction are labeled **10**–**18** in Table 2. Figure 2 shows the first step of the synthesis of Ruhemann's Purple, i.e., the reaction between the substituted ninhydrin analogues and alanine to form a substituted intermediate. These intermediates are labeled structures **19**–**27** in Table 3. Throughout this study all

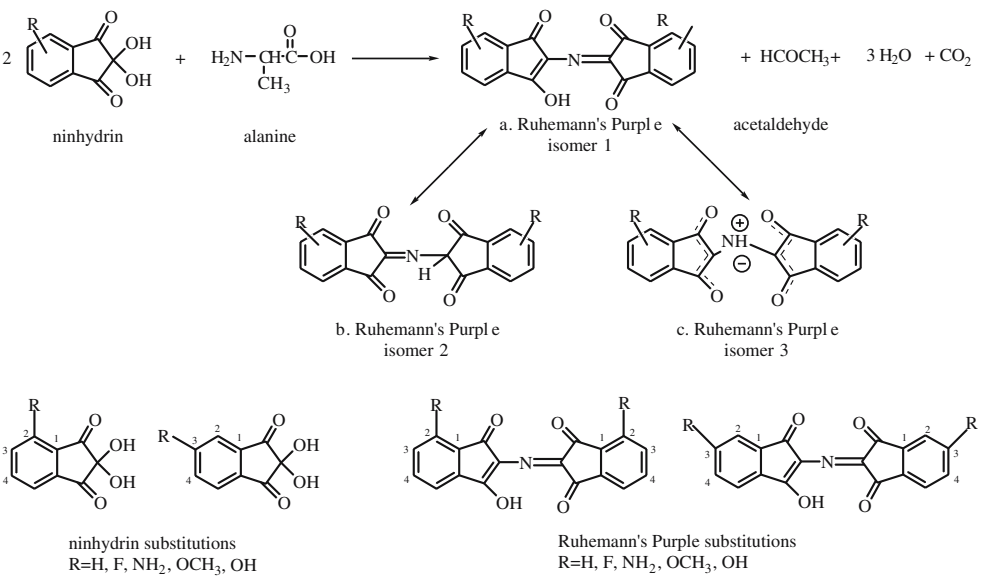


Fig. 1 The net reaction between alanine and the substituted ninhydrin analogues. All three isomers of Ruhemann's Purple discussed in this study are shown (*a*–*c*). The lower left hand corner shows the position numbers for the substitution of sidegroups (R=H, F, NH_2, OCH_3, OH) on the six-member ring of ninhydrin. If the side group is substituted at position C2 or C3 on ninhydrin then we considered only the produced Ruhemann's Purple that has both side groups substituted at the same positions on both its six-member rings

Table 1 Hartree–Fock theory 6-31G** energies for optimized structures of alanine, ninhydrin, its analogues, and its products with alanine

Structure[a]	HF 6-31G** energy (hartree)[b]
Ninhydrin (**1**)	−643.77216
Alanine	−321.88585
Acetaldehyde	−152.92265
Carbon dioxide	−187.63439
Water	−76.02361
Fluoroninhydrin (**2**)	−742.61561
Fluoroninhydrin (**3**)	−742.62015
Aminoninhydrin (**4**)	−698.81878
Aminoninhydrin (**5**)	−698.81054
Methoxyininhydrin (**6**)	−757.65593
Methoxyninhydrin (**7**)	−757.66307
Hydroxyninhydrin (**8**)	−718.64468
Hydroxyninhydrin (**9**)	−718.63453

[a] Even numbers in parenthesis indicate the side group (R=F, NH_2, OCH_3, OH), is substituted at the C3-position on the six member ring of ninhydrin. For odd numbers in parenthesis the substitution is at the C2- position on the six member ring of ninhydrin (cf. Fig. 1). Ninhydrin (**1**) is unsubstituted ninhydrin

[b] Note that 1 hartree=626.5 kcal mol^{-1}

Table 2 Hartree Fock Theory 6-31G** energies for optimized structures of Ruhemann's Purple and its analogues

Structure[a]	HF 6-31G** energy (hartree)
Ruhemann's Purple (**10**) isomer 1[b]	−1040.77428
Ruhemann's Purple isomer 2	−1040.787130
Ruhemann's Purple isomer 3	−1040.769185
Ruhemann's Purple-F (**11**)	−1238.46192
Ruhemann's Purple-F (**12**)	−1238.47031
Ruhemann's Purple-NH_2 (**13**)	−1150.85136
Ruhemann's Purple-NH_2 (**14**)	−1150.84773
Ruhemann's Purple-OCH_3 (**15**)	−1268.53424
Ruhemann's Purple-OCH_3 (**16**)	−1268.53907
Ruhemann's Purple-OH (**17**)	−1190.50446
Ruhemann's Purple-OH (**18**)	−1190.49527

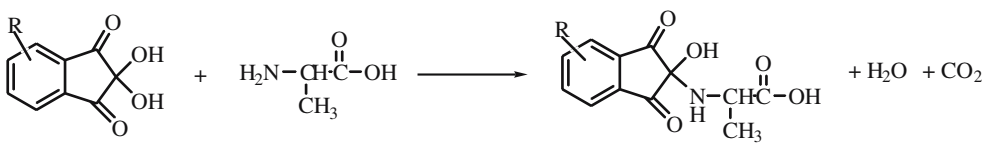
[a] Even numbers in parenthesis indicate the side group (R=F, NH2, OCH3, OH), is substituted at the C3-positions on the six member rings of Ruhemann's Purple. For odd numbers in parenthesis the substitution is at the C2- positions on the six member ring of Ruhemann's Purple (cf. Fig. 1). Ruhemann's Purple (**10**) is unsubstituted Ruhemann's Purple

[b] The Ruhemann's Purple isomer used in the reaction energetic analysis for this study (isomer 1 cf. Fig. 1a)

structures labeled with even numbers have their side groups substituted at the C3-position on the six-member ring of ninhydrin (cf. Fig. 1) and all structures labeled with odd numbers are substituted at the C2-position (cf. Fig. 1). For the Ruhemann's Purples, both six member rings have a single substitution at the same position (cf. Fig. 1).

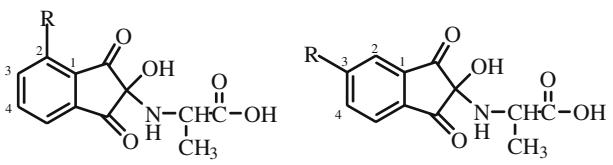
Tables 1, 2 and 3 list the energies of the Hartree-Fock (HF/6-31G**) optimized geometries of each compound shown in Figs. 1 and 2. Table 4 lists the net reaction energy (calculated as energy of the products minus energy of the reactants) required to form the substituted Ruhemann's Purple analogues. Finally, Table 5 gives the reaction energies of the initial reaction in the mechanism for the formation of the Ruhemann's Purple analogues.

The exact structure of Ruhemann's Purple that exists in solution or in solid phase (as in the case of fingerprint analysis) has been debated in the literature [23–27]. In particular, three different isomers have been identified or inferred by using different spectroscopic techniques. Figure 1 displays the three isomers of Ruhemann's purple in question. It has been argued by both experiment [23, 28] and theory [24] that the most brightly colored version of Ruhemann's purple is an anionic version of structure in *a*, where the anion stems from removing a proton from the



ninhydrin alanine first intermediate

Fig. 2 The first step of the synthesis of Ruhemann's Purple, i.e., the reaction between the substituted ninhydrin analogues and alanine to form the substituted first intermediate



intermediate substitutions
R=H, F, NH_2, OCH_3, OH

Table 3 Hartree Fock Theory 6-31G** energies for optimized structures of the unsubstituted intermediate and its substituted analogues

Structure[a]	HF 6-31G** energy (hartree)
Unsubstituted intermediate (**19**)	−889.62934
Intermediate -F (**20**)	−988.48016
Intermediate -F (**21**)	−988.47202
Intermediate -NH_2 (**22**)	−944.67066
Intermediate -NH_2 (**23**)	−944.66719
Intermediate -OCH_3 (**24**)	−1003.50711
Intermediate -OCH_3 (**25**)	−1003.51545
Intermediate -OH (**26**)	−964.49674
Intermediate -OH (**27**)	−964.49379

[a] Even numbers in parenthesis indicate the side group (R=F, NH2, OCH3, OH), is substituted at the C3-position on the six member ring of the intermediate. For odd numbers in parenthesis the substitution is at the C2- position on the six member ring of the intermediate

Table 5 Hartree Fock Theory 6-31G** reaction energies for the formation of the unsubstituted intermediate and its substituted analogues

Structure	Reaction energy (kcal mol^{-1})[a]
Unsubstituted intermediate (**19**)	3.14
Intermediate -F (**20**)	−1.45
Intermediate -F (**21**)	6.51
Intermediate -NH_2 (**22**)	6.50
Intermediate -NH_2 (**23**)	3.51
Intermediate -OCH_3 (**24**)	6.94
Intermediate -OCH_3 (**25**)	6.19
Intermediate -OH (**26**)	8.24
Intermediate -OH (**27**)	1.87

[a] Note, 1 hartree=626.51 kcal mol^{-1}

hydroxide group. Wigfield et al. however have argued that the protonated version of the colored anion is isomer *b* shown in Fig. 1b [23]. Using semi-empirical quantum calculations, Elber, Frank and Almog have confirmed the stability of a ylide form of Ruhemann's Purple (Fig. 1c) put forth by Grigg et al. from X-ray crystallographic data [18].

According to the computational predictions of Dietz et al., the most abundant isomer of Ruhemann's purple in an acidic environment (such as those under forensic field conditions) is likely the protonated version of the highly colored anion (Fig. 1a). In fact, ground and excited state computations of Dietz et al. also show that the electronic structures and spectra of isomers *a* and *c* are quite similar [24]. The structure of Dietz et al. (Fig. 1a), is the reference structure for Ruhemann's Purple we use in this study. Our reason for focusing on protonated versions of anionic Ruhemann's Purple is the fact that the ninhydrin-alanine reaction generally takes place in solution or on paper (solid phase) under somewhat acidic conditions [28]. Thus we believe that protonated versions of Ruhemann's Purple anions are the most abundant under standard reaction conditions. We have also carried out computational predictions of the structures and energetics of isomers *b* and *c* given by Wigfield et al. and Grigg et al. [23, 25, 26]. The electronic energies of Ruhemann's Purple isomers *a*, *b* and *c* (unsubstituted) appear in Table 2. Note that isomer *b* is 8.06 kcal mol^{-1} lower in energy than isomer *a*, and isomer *c* is 3.20 kcal mol^{-1} higher in energy than isomer *a*, respectively. Taken together, these three isomers of Ruhemann's Purple are all quite close in energy.

Experimentally inferred mechanistic schemes for the reaction of ninhydrin with amino acids have been given by several authors [29–37]. The mechanism of Ruhemann's purple formation, put forth by Johnson and McCaldin (and referred to as the McCaldin mechanism) is the earliest accepted scheme [31, 32]. The second, which we shall refer to as the Lamothe mechanism, represents essentially an extension of the McCaldin mechanism. It was given by Lamothe and McCormick in order to better account for the formation of hydrindantin, a reddish compound known to appear over the course of the reaction [34, 35]. Yet another mechanism was suggested by Friedman and Williams (the Friedman mechanism) using further experimentation [36]. This latter mechanism is a radical departure from those given by McCaldin and Lamothe. Recently, we used Hartree–Fock computations to postulate that in fact all three of these mechanisms are energetically feasible from a reaction surface point of view, and all are likely operable at elevated temperatures [29–37]. We have suggested a slight modification to the McCaldin mechanism as the operable mechanism of formation of Ruhemann's purple at room temperature (~25 °C) considering the relatively high endothermicities associated with some side reactions involved in the Lamothe and Friedman mechanisms.

Table 4 Hartree Fock Theory 6-31G** net reaction energies for the synthesis of Ruhemann's Purple and its analogues

Ruhemann's Purple substitution	Reaction energy (kcal mol^{-1})[a]
Unsubstituted Ruhemann's Purple	17.52
Ruhemann's Purple-F (**11**)	17.12
Ruhemann's Purple-F (**12**)	17.55
Ruhemann's Purple-NH_2 (**13**)	27.72
Ruhemann's Purple-NH_2 (**14**)	19.66
Ruhemann's Purple-OCH_3 (**15**)	22.34
Ruhemann's Purple-OCH_3 (**16**)	28.27
Ruhemann's Purple-OH (**17**)	26.91
Ruhemann's Purple-OH (**18**)	19.94

[a] Note, 1 hartree=626.51 kcal mol^{-1}

When considering total net energies consumed or released in the formation of Ruhemann's Purple, it is irrelevant which mechanism is considered. This is because we are not concerned with the details of what chemistry occurs between the starting material and products, only the energetic differences between them. All three mechanisms consume ninhydrin and alanine to produce Ruhemann's purple. Again, the net total reaction is shown in Fig. 1. As can be seen from Table 4, the net total reactions involving both substituted and unsubtituted Ruhemann's purple are endothermic. The substituted side groups tend to increase the endothermicity slightly in comparison to the unsubstituted reaction. The exception is when fluorine is substituted at the C2-position on the six-member ring of ninhydrin (Fig. 1); however, the decrease in endothermicity with respect to the unsubstituted reaction is only slight (~0.4 kcal mol^{-1}). The principal reason for the increase in endothermicity in the net total reaction seems to be steric in nature as can be observed from Table 4. In general, the bulkier the side group, the higher the endothermicity. All increases in endothermicity (with respect to the reaction with the unsubstituted species) are small, with the largest increase coming from the methoxy side group ($-OCH_3$) substituted at the C3-position on the six-member ring of ninhydrin (Fig. 1). We see this energetic trend for the substitution at the C2-position on the six-member ring of ninhydrin as well as for ninhydrins substituted at the C3-position. There is no clear relationship between the reaction energy and whether or not the side group is substituted at the C2- or C3-position on the six member ring.

The same general trends can be observed for the reactions involving both the substituted and unsubstituted intermediate (Table 5). The exception is for the –OH substitutions, which show both the largest (8.24 kcal mol^{-1}) and the smallest (1.87 kcal mol^{-1}) endothermicities. Also, an interesting result appears for the reaction of alanine and ninhydrin with fluorine substituted at the C3-position on the six-member ring of ninhydrin. This reaction is slightly exothermic (−1.45 kcal mol^{-1}). All the other reactions between alanine and ninhydrin (both substituted and unsubstituted) are endothermic, ranging from 1.87 kcal mol^{-1} (–OH substituted at the C2-position on the six-member ring of ninhydrin) to 8.24 kcal mol^{-1} (–OH substituted at the C3-position on the six-member ring of ninhydrin).

Conclusion

Our results show the possibility of finding an analog of ninhydrin that might undergo a less endothermic process as it forms (substituted) Ruhemann's Purple. It might be advisable for experimentalists to synthesize the fluorine-containing analog, as well as other similar molecules and determine their spectroscopic properties in order to examine if these compounds may be more satisfactory for the production of colored product when applied to fingerprints. The investigation of the activity of these molecules can lead to the discovery of better agents for fingerprinting. We are currently performing excited state computations on all of the species discussed in this study in order to help predict their (electronic) spectroscopic properties, in particular their ability to fluoresce.

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References

1. Almog J (2001) Fingerprint development by ninhydrin and its analogues. In: Lee HC, Gaensslen RE (eds) Advances in fingerprint technology, 2nd edn. CRC Press, New York, pp 177–210
2. Berry J, Stoney DA (2001) History and development of fingerprinting. In: Lee HC, Gaensslen RE (eds) Advances in fingerprint technology, 2nd edn. CRC Press, New York, pp 1–52
3. Friedman M (2004) J Agric Food Chem 52:385–415
4. Gaensslen RE, Young KR (2005) Fingerprints. In: James SH, Nordby JJ, (eds) Forensic science an introduction to scientific and investigative techniques. CRC, New York, pp 341–360
5. Margot P, Lennard C (1994) Fingerprint detection techniques, 6th edn. University of Lausanne, Lausanne
6. Lee HC, Gaensslen RE (2001) Methods of latent fingerprint development. In: Lee HC, Gaensslen RE (eds) Advances in fingerprint technology, 2nd edn. CRC, New York, pp 105–176
7. Saferstein RE (2006) Criminalistics: an introduction to forensic science, 9th edn. Prentice Hall, New York
8. Almog J (2001) Fingerprint development by ninhydrin and its analogues. In: Lee HC, Gaensslen RE (eds) Advances in fingerprint technology, 2nd edn. CRC, New York, pp 177–210
9. Ruhemann S (1910) Trans Chem Soc 97:1438–1452
10. Ruhemann S (1910) Trans Chem Soc 97:2025–2036
11. Ruhemann S (1911) Trans Chem Soc 99:792–812
12. Ruhemann S (1911) Trans Chem Soc 99:1306–1311
13. Ruhemann S (1911) Trans Chem Soc 99:1486–1491
14. Ruhemann S (1912) Trans Chem Soc 101:780–795
15. Pounds CA (1988) Developments in fingerprint visulization. In: Maehly A, Williams RL (eds) Forensic science progress. Springer, Berlin Heidelberg New York, pp 91–119
16. Pounds CA, Grigg R, Mongkolaussavaratana T (1990) J Forensic Sci 35:169175
17. Menzel ER (1999) Fingerprint detection with lasers, 2nd edn. Dekker, New York
18. Elber R, Frank A, Almog J (2000) J Forensic Sci 45:757–760
19. Petraco NDK, Proni G, Jackiw JJ, Sapse A-M (2006) J Forensic Sci 51:1267–1275
20. Hehre WJ, Kong J (2004) Spartan '04 windows. Wavefunction, Irvine, CA

21. Hehre WJ, Ditchfield R, Pople JA (1972) J Chem Phys 56:2257–2291
22. Hariharan PC, Pople JA (1973) Theor Chim Acta 28:213–222
23. Wigfield DC, Buchanan GW, Croteau SM (1980) Can J Chem 58: 201–208
24. Dietz F, Rommel-Mohle K, Schleitzer A, Tyutyulkov N (1993) Z Naturforsch 48b:1133–1137
25. Grigg R, Malone JF, Mongkolaussavaratana T, Thianpatanagul S (1989) Tetrahedron 45:3849–3862
26. Grigg R, Malone JF, Mongkolaussavaratana T, Thianpatanagul S (1986) J Chem Soc Chem Commun 1986:421–422
27. Pine SH, Hendrickson JB, Cram DJ, Hammond GS (1980) Organic chemistry, 4th edn. McGraw-Hill, New York
28. Joullie MM, Thompson TR, Nemeroff NH (1991) Tetrahedron 47: 8791–8882
29. MacFayden DA, Fowler NJ (1950) J Biol Chem 186:13–22
30. Schonberg A, Moubasher R (1952) Chem Rev 50:261–294
31. Johnson AW, McCaldin DJ (1958) J Chem Soc 158:817
32. McCaldin DJ (1960) Chem Rev 60:39–50
33. Wittmann H, Muller AK, Ziegler E (1969) Monatsh Chem 100: 405–497
34. Lamothe PJ, McCormick PG (1972) Anal Chem 44:821–845
35. Lamothe PJ, McCormick PG (1972) Anal Chem 45:1906–1916
36. Friedman M, Williams LD (1974) Bioorg Chem 3:267–283
37. Schertz TD, Reiter RC, Stevenson CD (2001) J Org Chem 66: 7596–7603