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Forensic Applications of X-Ray Diffraction. I: Differentiation of Piperidyl Benzilates and Related Glycolates by Micro-X-Ray Diffraction

The differentiation of closely related compounds is a subject of considerable forensic importance, especially for drugs where relatively minor compositional or structural variations, such as different substituents or isomerism, have considerable physiological effects and may distinguish between a controlled substance and an uncontrolled one. Frequently, common techniques such as ultraviolet (UV) spectroscopy are ineffective in such cases [*1*], and others such as thin-layer chromatography (TLC) [*2*] lack specificity of response. Some effective techniques such as mass spectrometry (MS) [*3*] are often not readily available.

In this study, the use of powder X-ray diffraction (XRD) [*4,5*] in a situation of this type is explored. Forensic use of powder XRD methods has increased [*6,7*] because XRD is a definitive determinative technique (in conjunction with chemical analysis, where required), is nondestructive, and can be used on extremely small sample quantities of as little as 0.1 μg [*8*]. If the latter aspect is important, there is an advantage in using the Gandolfi camera [*9*], which has been introduced recently in several forensic laboratories [*7*].

The Gandolfi camera was originally designed to permit recording of a powder pattern from a single crystal by simultaneous sample rotation about two axes, one of which is the usual rotation axis of a Debye-Scherrer camera while the other is inclined to it at an angle of 45 deg. When powders are examined, the Gandolfi camera can produce sharp and continuous lines from a smaller sample than can a Debye-Scherrer camera. A recent discussion of forensic use of the Gandolfi camera has been given by Canfield and DeForest [*7*].

The compounds considered here are piperidyl benzilates having various substituents of the glycolic acid group [*10,11*]. These compounds have hallucinogenic activity and two of them are classified under Drug Enforcement Administration (DEA) Schedule I. Their identification is thus of forensic interest. Petersen and co-workers have recently shown [*3*] that MS is a powerful technique for the identification of these compounds and provides ready differentiation; additionally, previous methods for their differentiation were reviewed [*3*].

We have determined the X-ray powder diffraction patterns of ten of these compounds made available to us by the DEA (Table 1). The powder patterns of two of these,

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TABLE 1—*Piperidyl benzilates and related glycolates studied by Gandolfi XRD.*

	Compound	Formula	Commercial Code Number	Remarks
A.	*N*-ethyl-3-piperidylbenzilate	$C_{12}H_{25}NO_3$	JB318	also reported by Folen [*6*]
B.	*N*-methyl-3-piperidylbenzilate HCl	$C_{20}H_{24}NO_3Cl$	JB336	also reported by Folen [*6*]
C.	*N*-methyl-4-piperidylbenzilate	$C_{20}H_{23}NO_3$	JB8191	...
D.	3-piperidylbenzilate	$C_{19}H_{21}NO_3$	JB841	...
E.	*N*-ethyl-3-piperidyldiphenylacetate	$C_{21}H_{25}NO_2$	JB305	...
F.	*N*-ethyl-3-piperidylcyclopentyl-glycolate	$C_{20}H_{29}NO_3$	JB478	...
G.	*N*-methyl-3-piperidylphenylcyclo-hexylglycolate	$C_{20}H_{29}NO_3$	JB840	...
H.	*N*-allyl-3-piperidylbenzilate	$C_{22}H_{25}NO_3$	JF18	...
I.	*N*-cinnamyl-3-piperidylbenzilate	$C_{28}H_{29}NO_3$	JB8008	...
J.	*N*-(dimethylaminoethyl)-3-piperi-dylbenzilate	$C_{23}H_{30}N_2O_3$	JB851	...

JB318 and JB336, have previously been reported in a published listing of the powder patterns of 73 drugs, excipients, and adulterants [*6*].

Experimental Methods

Patterns were recorded from powder samples, typically weighing about 10 μg, which were mounted near the tip of a glass fiber. The fiber, of about 0.1 mm diameter, had been coated with a thin layer of petrolatum to promote adhesion of the powder.

For decreased exposure time, the small diameter (57.3 mm) Gandolfi camera was used; CrK_α radiation was used to increase resolution. Exposure times of 5 to 8 h were employed with Ilford Industrial G X-ray film. Films were measured both visually, by using a light box and a vernier caliper, and with a recording microdensitometer (Jarrell-Ash 23-500). The 2θ values given in Table 2 are from the vernier caliper measurements; the relative intensities I/I_1 were measured on the densitometer trace with guidance from visual characterization for the weaker lines.

Line diagrams of the 2θ values were plotted with the computer program CAIN, written by Abel and Kemmey [*12*] and modified by one of us (J. C. B). This program also stores the patterns in a data file and contains a search routine which accepts an unknown input pattern and searches the file to match the unknown against the known patterns.

Results

The compounds examined in this study, with their chemical formulas and commercial code designations, are listed in Table 1. Table 2 (*A–J*) presents the powder patterns obtained in this study. Figures 1 *A* to *J* show the line diagrams of these patterns.

Literature data [*6*] for JB318 and JB336 are included for comparison (*K* and *L* in Table 2 and Fig. 1); these data had been obtained by use of diffractometry on samples larger by factors of 10^2 to 10^3 than the present ones and show greater resolution. The 2θ values shown for Folen's data [*6*] are calculated from his interplanar spacings (*d* values) as those which would be observed using CrK_α radiation in order to ease comparison with the results of the present work.

TABLE 2—*Complete powder diffraction data (2θ values are for CrK_α X-radiation).*

2θ	d, Å[a]	I/I_1	2θ	d, Å[a]	I/I_1
	A. JB318		65.0	2.132	10
			68.0	2.048	8
11.35	11.6	52	70.0	1.997	8
14.0	9.4	8	71.45	1.962	10
16.2	8.11	52	74.0	1.903	8
17.0	7.74	51	78.9	1.802	10
18.3	7.21	4			
20.0	6.61	25			
23.0	5.74	10		C. JB8191	
25.25	5.24	100			
26.0	5.085	84	14.4	9.12	30
31.2	4.26	22	15.8	8.33	62
33.9	3.93	48	17.4	7.59	21
36.1	3.70	68	20.0	6.58	56
37.5	3.56	63	22.4	5.89	77
38.0	3.52	46	26.9	4.925	49
39.7	3.38	28	28.5	4.653	49
41.8	3.21	4	29.1	4.555	49
43.8	3.068	12	30.8	4.319	100
44.8	3.004	10	32.0	4.146	31
46.9	2.878	30	33.2	4.007	23
49.5	2.735	21	35.9	3.717	10
53.0	2.57	6	37.0	3.612	31
55.2	2.47	11	41.2	3.258	23
57.8	2.37	22	43.3	3.107	31
60.8	2.263	15	46.0	2.930	15
62.3	2.215	7	48.6	2.783	20
70.1	1.995	7			
72.3	1.943	11		D. JB841	
76.9	1.848	11			
79.8	1.786	6	11.2	11.7	81
			14.9	8.83	85
	B. JB336		18.3	7.21	97
			20.3	6.49	100
14.35	9.17	65	20.8	6.35	30
16.3	8.10	55	22.35	5.91	79
17.8	7.42	66	23.5	5.63	24
19.7	6.68	76	24.5	5.39	74
21.8	6.06	40	26.2	5.05	65
24.4	5.41	40	27.5	4.82	42
26.3	5.04	70	28.1	4.71	71
28.0	4.73	14	29.6	4.485	58
30.15	4.40	44	31.0	4.29	21
32.7	4.07	100	33.0	4.03	24
34.3	3.88	10	34.3	3.883	81
36.2	3.687	29	35.5	3.753	20
38.0	3.518	16	37.0	3.609	78
39.7	3.371	44	38.35	3.487	7
41.9	3.201	40	40.0	3.348	25
44.5	3.023	25	41.7	3.219	22
48.1	2.809	35	44.5	3.027	14
49.5	2.736	35	45.85	2.941	14
53.1	2.562	10	47.1	2.867	14
54.0	2.523	10	49.9	2.718	30
57.7	2.373	15	51.2	2.650	14
59.6	2.306	10	53.0	2.569	14
61.9	2.227	10	54.5	2.503	18
63.7	2.171	10	58.0	2.359	12

TABLE 2—*Continued.*

2θ	d, Å[a]	I/I_1	2θ	d, Å[a]	I/I_1
61.4	2.243	11	38.8	3.452	34
64.5	2.1475	11	41.7	3.222	12
67.2	2.071	7	43.3	3.104	12
69.2	2.017	7	46.0	2.927	18
72.5	1.938	11	48.3	2.80	8
77.4	1.832	6	50.4	2.688	6
79.7	1.777	7	60.7	2.266	6
			63.0	2.190	8
	E. JB305		64.95	2.133	6
13.1	10.08	55			
14.0	9.39	52		G. JB840	
16.4	8.04	70	14.7	8.9	40
20.0	6.60	38	15.8	8.33	60
22.25	5.94	100	17.5	7.52	65
24.1	5.49	87	18.8	7.01	62
26.3	5.04	46	21.95	6.02	100
27.2	4.87	35	24.0	5.50	50
28.2	4.71	13	24.7	5.36	70
29.5	4.50	58	25.7	5.14	47
30.6	4.34	53	26.7	4.96	65
32.5	4.10	12	28.35	4.68	15
33.2	4.01	30	29.1	4.56	13
34.8	3.83	20	30.4	4.365	76
36.45	3.66	38	31.4	4.23	50
38.5	3.48	35	32.2	4.13	50
40.2	3.33	70	35.9	3.713	40
41.2	3.25	15	38.0	3.512	50
44.2	3.045	37	38.9	3.443	25
46.15	2.922	39	40.2	3.330	15
48.2	2.806	15	42.75	3.143	18
52.5	2.59	19	44.5	3.023	19
54.1	2.518	28	46.4	2.906	10
58.0	2.363	30	48.7	2.777	20
61.5	2.239	14	50.4	2.689	20
63.3	2.184	14	52.9	2.572	18
65.25	2.125	14	58.65	2.339	20
67.3	2.066	14	62.0	2.224	6
69.6	2.007	7	64.0	2.162	6
72.41	1.939	10	67.1	2.074	10
74.4	1.894	7			
				H. JF18	
	F. JB478		11.0	11.9	45
11.55	11.4	85	14.6	9.01	90
14.9	8.83	100	17.6	7.49	85
17.6	7.48	32	19.4	6.80	40
19.15	6.89	80	21.0	6.28	30
23.0	5.74	30	21.6	6.10	30
24.0	5.51	30	23.4	5.64	45
26.0	5.09	32	24.8	5.34	44
27.7	4.78	80	26.6	4.98	56
29.7	4.47	32	27.7	4.78	56
31.1	4.27	34	29.1	4.55	64
32.1	4.14	27	32.1	4.15	38
35.0	3.81	34	33.0	4.037	45
37.2	3.595	26	35.8	3.731	100

TABLE 2—*Continued.*

2θ	d, Å[a]	I/I_1	2θ	d, Å[a]	I/I_1
37.1	3.597	64		K. JB318 [6]	
39.1	3.422	24	11.43	11.5	39
41.0	3.271	45	13.99	9.40	15
43.05	3.122	16	14.49	9.08	19
44.8	3.004	15	15.48	8.50	20
46.8	2.887	11	16.83	7.82	100
49.2	2.752	15	19.73	6.68	26
52.1	2.610	17	20.61	6.40	20
53.7	2.536	8	23.01	5.74	14
55.7	2.453	10	24.80	5.33	40
58.6	2.342	15	25.14	5.26	66
61.95	2.226	14	25.79	5.13	57
64.8	2.139	6	26.52	4.99	57
67.2	2.070	8	31.14	4.26	45
			32.59	4.08	12
	I. JB8008		34.05	3.91	66
			36.04	3.69	68
11.0	11.9	100	37.08	3.59	43
18.9	6.97	41	37.96	3.52	51
20.45	6.45	38	39.47	3.39	22
22.0	6.01	36	43.64	3.08	9
24.0	5.50	10	44.71	3.01	9
26.4	5.02	70	46.96	2.873	22
27.4	4.84	58	47.66	2.835	9
29.1	4.56	21	48.60	2.782	8
30.2	4.39	27	49.55	2.732	14
31.8	4.18	13	55.27	2.468	7
32.7	4.07	38	58.04	2.360	8
36.9	3.62	38	70.04	1.995	3
38.5	3.48	12	72.33	1.940	5
41.0	3.27	38	76.70	1.845	3
45.7	2.95	12			
48.2	2.805	15		L. JB336 [6]	
49.0	2.761	8	14.63	8.97	47
54.2	2.515	8	16.25	8.1	37
59.6	2.304	8	17.83	7.37	53
64.4	2.151	6	19.53	6.75	57
			21.85	6.04	35
	J. JB851		24.48	5.4	33
			25.29	5.23	14
15.6	8.46	100	26.10	5.07	45
23.7	5.58	77	28.19	4.70	20
26.2	5.06	16	29.48	4.5	31
28.0	4.73	45	30.66	4.33	31
29.3	4.53	40	32.18	4.13	43
35.3	3.78	10	32.59	4.08	100
37.4	3.57	15	36.15	3.69	29
38.1	3.507	40	37.84	3.53	16
38.8	3.449	15	39.72	3.37	43
41.3	3.248	13	41.92	3.2	18
44.1	3.053	8	43.64	3.08	16
46.5	2.904	13	44.55	3.02	20
48.3	2.801	13	47.90	2.82	18
51.1	2.656	8	49.20	2.75	20

[a] 1 Å = 0.1 nm

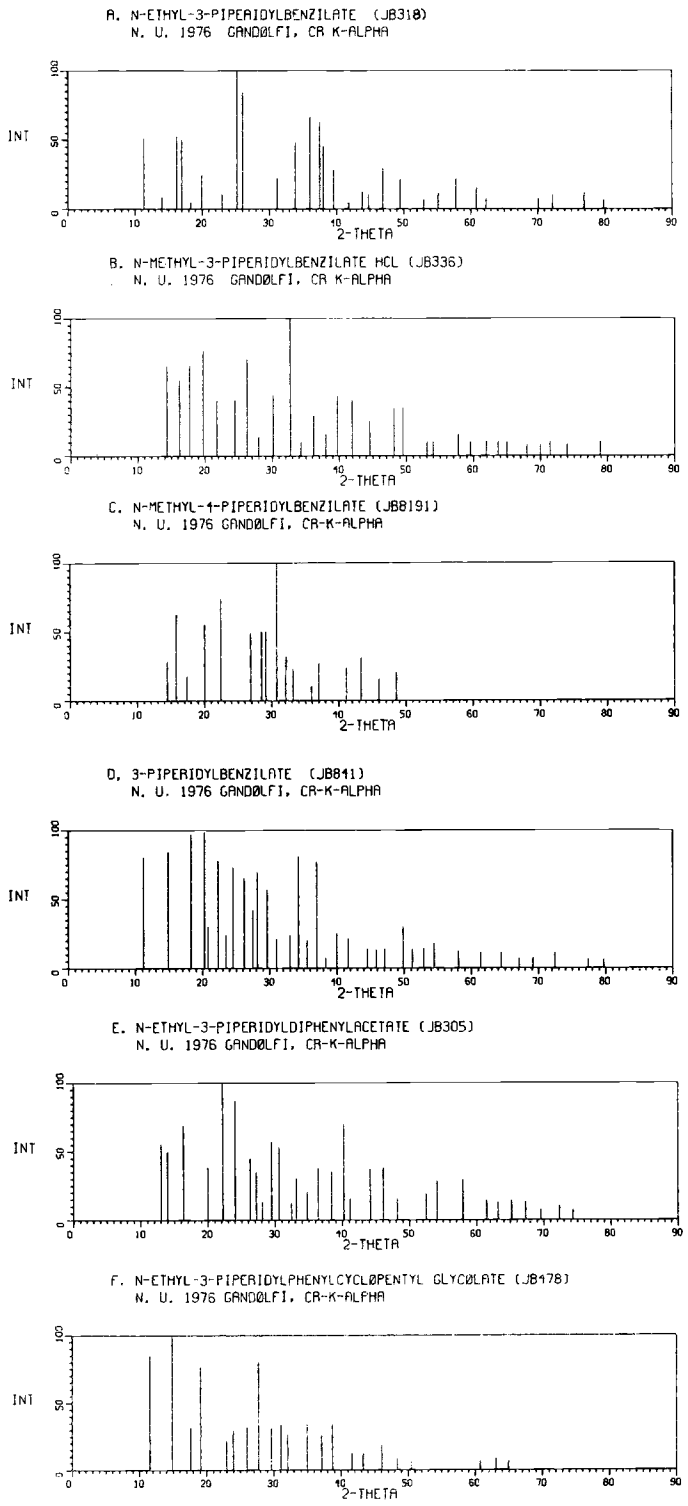

FIG. 1—A *through* F: *Line diagrams of powder patterns.*

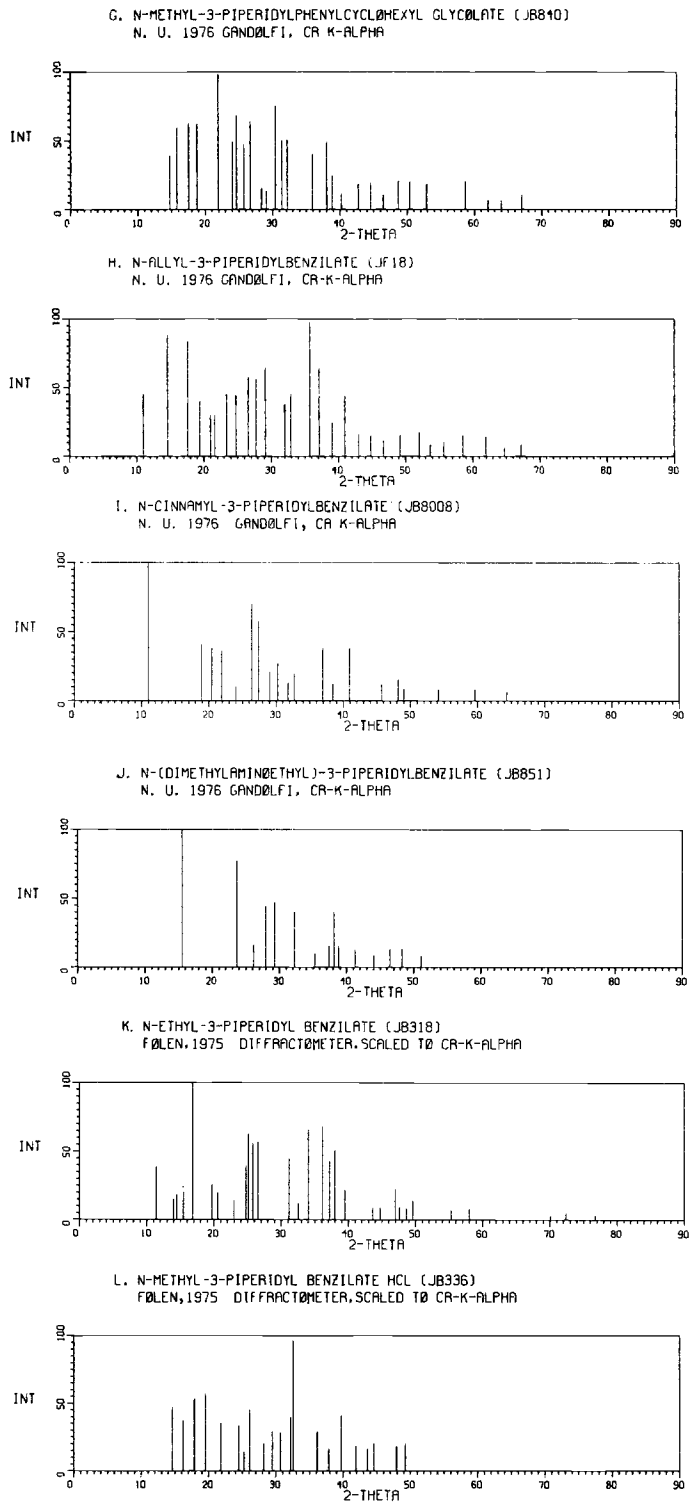

FIG. 1—G *through* L: *Line diagrams of powder patterns.*

Discussion

The present patterns have a maximum 2θ resolution of approximately 0.5 deg, adequate for differentiation among the compounds studied. This gives rise to differences between the Gandolfi patterns and those of Folen [6], which have higher resolution, wherein closely spaced peaks may or may not have been resolved. For example, for JB336, we show one line at about 30 deg, while Folen has two lines bracketing 30 deg; our strongest line at 32.7 deg is also shown by Folen as a doublet. Similarly, for JB318, we show one line at about 20 deg, while Folen shows a doublet; our two lines in the vicinity of 25 and 26 deg are shown by Folen as four lines; our line at about 13 deg (appearing as very broad in our densitometer trace) is shown by Folen as three lines; conversely, our doublet in the vicinity of 16 and 17 deg is shown by Folen as his strongest single line.

The other noticeable differences arise from the inclusion of weaker lines in one pattern but not in the other. For example, for JB336, Folen shows a weak line at about 25.2 deg, while we did not; we detect a line at about 34.2 deg, while Folen did not. For JB318, we detect a line at 18.2 deg, while Folen did not; we did not detect the line at 32.5 deg shown by Folen.

Taking account of the differences noted above, it can be concluded that the two pairs of patterns for JB336 and JB318 do match; no peak of more than weak intensity is present in one pattern and not in the other.

Nevertheless, because of the potentially different assignment of the peaks of maximum intensity, for example, as shown for JB318, it is possible that difficulties could be encountered in establishing a match when starting with the Gandolfi pattern as an unknown and searching the literature on the basis of the strongest lines. This demonstrates the desirability of considering relative resolution when comparing patterns. Computer searching in which all lines, rather than just the strongest ones, are used can overcome this problem.

In comparing the different readout methods, we found that the visual method was faster and adequately accurate, compared to the microphotometric method, for the purpose of line position analysis. In fact, for weak lines, higher angle lines, and overlapping lines, the visual method is superior to the photometric one since the densitometer is more adversely affected than the eye by the statistical background introduced by film graininess; that is, the eye is a more efficient integrator. However, the photometric method is more precise and is therefore preferable for intensity measurements and line position analysis of highest precision.

Comparison of the ten new patterns shows that all of them can be distinguished from one another despite the close chemical relationships of the compounds. All the compounds appear to have substantially different unit cells; small chemical differences are thus magnified in the crystal structures and the resulting diffraction patterns for these samples.

As these results were obtained on very small samples, they appear to have practical potential for forensic application. It was noted that the ordinary Debye-Scherrer powder patterns obtained from the same samples were very spotty and not adequate for obtaining a satisfactory determination of line positions and intensities.

In any application of the Gandolfi camera to drugs, it would be desirable to increase the resolution, for example by monochromatizing the radiation or increasing the camera diameter.

Summary

1. X-ray powder diffraction patterns of microgram quantities of drugs can be satisfactorily recorded with a Gandolfi camera.

2. Despite the chemical similarity of the drugs investigated, the XRD powder patterns of the present study differed substantially enough to make visual comparisons adequate to distinguish among them; this implies that micro-XRD powder patterns can be useful in establishing identity of drugs in more general cases.

3. Because of its ready availability and relatively inexpensive operation, powder XRD compares well with MS methods of drug analysis for samples as small as microgram quantities.

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